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(71) Applicant: EMORY UNIVERSITY [US/US]; 2009 Ridgewood Drive, Atlanta, GA 30322 (US).

(72) Inventor: DELAFONTAINE, Patrick; 2060 Deborah Drive, Atlanta, GA 30345 (US).

(74) Agents: GREENLEE, Lorance, L. et al.; Greenlee and Winner, Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).

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(54) Title: METHODS FOR REGULATION OF INSULIN-LIKE GROWTH FACTOR I RECEPTOR

(57) Abstract

The present invention provides nucleotide sequences and methods for increasing expression of type I insulin-like growth factor receptor, in particular to promote healing of burns, broken bones, and other wounds, nerve regeneration in injured tissue and angiogenesis. Inhibition of angiogenesis and/or vascular smooth muscle cell growth and proliferation can be achieved using IGF IR-specific antisense RNAs or oligonucleotides. IGF IR ATG-directed sense oligonucleotides elevate the expression of the IGF IR gene.

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METHODS FOR REGULATION OF INSULIN-LIKE GROWTH FACTOR I RECEPTOR

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Field of the Invention

The present invention relates to the use of oligonucleotide-mediated methods for the regulation of growth factor receptor gene expression, particularly insulin-like growth factor 1 receptor, and methods for wound healing, promoting blood vessel proliferation, preventing postangioplasty restenosis and/or post transplant atherosclerosis, and regulating smooth muscle cell growth.

Background of the Invention

Insulin-like growth factor I (IGF I) is a ubiquitous peptide which regulates growth and differentiation in many cell types. IGF 1, which is synthesized and secreted by vascular smooth muscle cells, acts as an autocrine/paracrine factor. IGF 1 acts to stimulate progression of cells from the G1 phase to the S phase of the cell cycle. Thus, IGF 1 serves a critical role in cell growth and proliferation and in normal development.

The effects of IGF are mediated by the type 1 IGF receptor (IGF IR), which is a membrane tyrosine kinase (Ullrich et al. (1986) *EMBO J.* 5:2503-2512; Werner et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:7451-7455). The IGR IR is a glycosylated heterotetramer which consists of two extracellular α subunits and

two transmembrane ß subunits which each have extracellular and intracellular portions. The α subunits contain the growth factor binding site. Several growth factors including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and Angiotensin II (Ang II) upregulate vascular smooth muscle cell (VSMC) IGF IR (Pfeile et al. (1987) *Endocrinology*, 120:2251-2258; Ververis et al. (1993) *Circulation Research*, 72:1285-1292). Pre-incubation of VSMC with bFGF leads to increased mitogenic responsiveness to IGF I (Ververis et al. (1993) supra). It has been shown that antisense targeting of the IGF IR inhibits growth of SV-40 transformed BALB/c3T3 fibroblasts (Porcu et al. (1992) *Mol. Cell. Biol.* 12:5069-5077). Furthermore, overexpression of the IGF IR has been shown to induce transformation of NIH/3T3 cells (Kaleko et al. (1990) *Mol. Cell. Biol.* 10:464-470).

Anti-IGF antiserum inhibits PDGF-mediated growth of VSMC (Clemmons et al. (1988) *J. Clin. Invest.* 75:1914-1918). Angiotensin II (Ang II), a vasoactive and mitogenic peptide, transcriptionally regulates the IGF I gene in VSMC, and neutralization of extracellular IGF I with an anti-IGF I antibody inhibits ang II-induced DNA synthesis in VSMC (Delafontaine et al. (1993) *J. Biol. Chem.* 268:16866-16890).

Summary of the Invention

The present invention provides methods for regulating cell growth, in particular vascular smooth muscle cell growth, and methods for stimulating proliferative process including, but not limited to, wound healing, burn healing, bone healing, nerve regeneration, and angiogenesis. Negative regulation of vascular smooth muscle cell growth and proliferation allows for the prevention or treatment of medical conditions including, but not limited to, coronary artery disease, post-angioplast restenosis, post coronary transplant atherosclerosis, atherosclerotic lesions and vascular complications of hypertension, including hypertensive retinopathy, hypertensive nephropathy, cerebrovascular disease and myocardial hypertrophy, and in the treatment of solid cancers.

Negative regulation of cell proliferation in particular of vascular smooth muscle cells is achieved using antisense oligonucleotides, which oligonucleotides are at least about 14 nucleotides, and preferably about 18 to about 22 nucleotides, in length and comprises contiguous sequence complementary to at least a portion of the mRNA encoding a growth factor receptor, for example, type I insulin-like growth factor receptor (IGF IR). Preferably, the antisense oligonucleotide, is complementary to the growth factor receptor mRNA sequence in the vicinity of the translation start site of the coding sequences. However, other antisense sequences complementary to coding sequence information is effective for inhibiting factor receptor gene expression, thereby inhibiting target cell growth and proliferation. Antisense nucleic acid molecules can also be made as transcription products from non-naturally occurring recombinant nucleic acid molecules expressed in the target tissue. Preferably, expressed antisense RNAs are at least about 100 nucleotides in length and up to 1 Kb or more. Specifically exemplified IGF IR antisense oligonucleotides useful in the present methods include those having sequences as given in SEQ ID NO:1 (ATG-directed) and SEQ ID NO: 4. Both these sequences and other antisense molecules complementary to IGF IR mRNA effectively inhibit IGF IR expression in rat and human cells, such as vascular smooth muscle cells, thereby preventing or ameliorating post angioplasty restenosis and post-transplant atherosclerosis of the coronary arteries, and inhibiting angiogenesis in solid tumors.

Another aspect of the present invention is the positive regulation of growth factor receptor gene expression via ATG-directed sense oligonucleotides and, as specifically embodied herein, for up-regulation of type I insulin-like growth factor receptor gene expression, with the result that cell growth and proliferation is stimulated. Thus, improved wound healing, burn healing, healing of broken bones, angiogenesis, nerve regeneration and the like is achieved when ATG-directed oligonucleotides are applied to or expressed in target tissue. Within this context, the target tissue can be wounded or burned

tissue, a broken bone, a damaged nerve, an area of p ripheral vascular disease, or cardiac tissue damaged by myocardial infarction. In the latter two examples, angiogenesis (development of collateral blood vessels) will ameliorate the damage. Within the scope of this aspect of the invention are ATG-directed sense oligonucleotides effective for stimulating growth factor receptor gene expression and, as specifically exemplified, an oligonucleotide comprising a sequence as given in SEQ ID NO: 2 is effective in human and rat cells and tissue. Functionally equivalent ATG-directed sense oligonucleotides can be readily produced, without the expense of undue experimentation, for other species using the teachings of the present disclosure taken with knowledge and techniques readily accessible to the art.

In the present methods based on inhibition of IGF IR expression, the antisense oligonucleotides can be chemically synthesized from ribonucleotide or deoxynucleotide precursors or they may be transcription products expressed from non-naturally occurring recombinant DNA molecules such as adenovirus or Epstein-Barr virus vectors. Preferably, such vectors do not persist in the tissues for very extended periods of time.

Preferred chemically synthesized sense or antisense oligonucleotides are those with chemical modifications which prolong persistence of intact molecules inside and outside cells, including, but not limited to, phosphorothioate, phosphorodithioate, phosphotriester, methylphosphonate, phosphoramidite, α-anomer and phosphoroselenoate oligonucleotides, the syntheses of which are known to the art.

Brief Description of the Figures

Figure 1 illustrates the effects of IGF IR ATG-directed oligonucleotides on basal and serum-induced DNA synthesis. VSMC were incubated in serum-free medium alone (SFM) or in the presence of antisense (AS), mismatch (M), or sense (S) oligonucleotides (SEQ ID Nos:1, 3 and 2, respectively) (0.1-10 uM) for 48 hr. [^{3}H]-Thymidine incorporation was then determined as basal levels (in the continued presence of SFM) and in response

to 10% calf serum (10% CS) as described in Example 3. Results are the mean ± standard error (SE) of duplicate determinations from three to ten independent experiments for each condition.

Figure 2 illustrates the effects of IGF IR ATG-directed oligonucleotides on the proliferation of VSMC. 50% confluent VSMC were exposed to SFM, DMEM with 10% CS alone, or DMEM with 10% CS and increasing concentrations of AS, M or S oligonucleotides (SEQ ID NOS:1, 3 and 2, respectively) (0.1 - 10 uM). Cell counts were determined at 96 hr. Shown is the mean ± SE of duplicate measurements from four separate experiments.

Figure 3 illustrates the effects of IGF IR oligonucleotides on the mitogenic response to IGF I. VSMC were incubated for 48 hr in DMEM containing 10% CS alone or in the presence of 5 uM AS or S oligonucleotides (SEQ ID NOS: 1 and 2, respectively). Cells were then exposed to fresh SFM containing increasing concentrations of human recombinant IGF I (0 - 50 ng/ml) for 24 hr and [^{3}H]-thymidine incorporation determined as described in Example 3. Results are the mean ± SE of duplicate determinations from four separate experiments.

Figure 4 illustrates the effects of IGF IR oligonucleotides on mitogenic response to ang II. VSMC were incubated for 48 hr in DMEM containing 10% CS alone or in the presence of 5 uM AS, M or S oligonucleotides (SEQ ID NOS: 1, 3 and 2, respectively). Cells were then exposed to fresh SFM containing increasing concentrations of ang II (0 - 1000 nM) for 36 hr. [^{3}H]-thymidine incorporation was determined as described in Example 3. Results are the mean ± SE of duplicate measurements from three to seven experiments for each condition.

Figures 5A-5C illustrate the effects of ATG-directed IGF IR oligonucleotides on IGF IR and Angiotensin II (Ang II) receptor numbers. Vascular smooth muscle cells (VSMC) were incubated in SFM (Fig. 5A) or in DMEM with 10% CS (Figs. 5B-5C) alone (control), or in the presence of antisense (AS), missense (M) or sense (S) oligonucleotides (SEQ ID Nos. 1, 3 or 2, respectively) (5μM) for 48 hours ^{125}I-IGF I (Figs 5A, 5B) and [^{125}I-Sar^1-Ile^8]-Ang II (Fig. 5C) displacement binding experiments were performed and data analyzed using the LIGAND program. Results shown are the

mean ± (SE) of duplicate determinations from two to six separate experiments for each condition.

Figures 6A-6B illustrate the effects of non-ATG-directed sense (S) and antisense (AS) oligonucleotides (SEQ ID Nos. 5 and 4, respectively) on VSMC DNA synthesis and IGF IR number. In Fig. 6A, VSMC were incubated in SFM alone or in the presence of AS or S oligonucleotides (0.1 - 10 μM) for 48 hours. [^{3}H]-thymidine incorporation was then determined basally (in the continued presence of SFM) or in response to 10% CS as described in Example 6. Results are the mean ± standard error of duplicate measurements from three to eight experiments for each condition. In Fig. 6B, VSMC were incubated in 10% CS alone (control), or in the presence of 5μM AS or S oligonucleotide for 48 hours, and radiolabeled IGF I binding experiments were performed as described in Example 8. Results are the mean ± SE of duplicate measurements from two separate experiments for each condition.

Figure 7 illustrates the effects of ATG-directed IGF IR antisense (AS), sense (S) and mismatch (M) oligonucleotides on IGF IR mRNA levels. VSMC were incubated for 48 hours in 10% CS alone (control) or in the presence of 5μM AS, M or S ODNs. Total RNA was extracted and co-hybridized using [^{32}P]-UTP labeled IGF IR and GAPDH antisense riboprobes. IGF IR mRNA levels (corrected for GAPDH) are shown as percent control (mean ± SE, n=5).

Figure 8 diagrammatically illustrates the construction of episomal vector pAnti-IGF IR. The rat IGF IR cDNA clone p118 was digested by XhoI and KpnI and the 0.8 kb restriction fragment was ligated in an antisense orientation into pCEP4. CMV, cytomegalovirus immediate early gene enhancer/promoter; SVpA, simian virus 40 poly A; EBNA-1, EBV-encoded nuclear antigen 1; oriP, EBV origin of replication; Hyg, hygromycin resistance gene; Amp, ampicillin resistance gene; Km, kanamycin resistance gene.

Figure 9A-9B illustrate the effect of antisense IGF IR cDNA transcription on IGF IR number and on DNA synthesis. Figure 9A shows IGF IR number per 10^5 cells in CA9 antisense-expressing and control cells. Confluent and up to two days postconfluent monolayers of clone CA9 (transfected with pAnti-IGF IR, CA) and of clones ME8 and ME10 (transfected with vector alone, ME)

underwent ^{125}I-IGF I radioligand binding studies as described in Example 6 herein. Binding data were analyzed using the LIGAND program. Shown is the mean ± SE of results from 8 separate experiments confluent and postconfluent monolayers of clone CA9 and clones ME8/ME10 were incubated in serum-free medium in the presence of 1 μCi/ml [^{3}H] thymidine for 24 hr and incorporated counts were determined as described in Example 8 herein. The means ± SE of triplicate determinations from 7 separate experiments are shown.

Figure 10 illustrates the effect of antisense IGF IR cDNA transcription on VSMC proliferation. Growth of cells of clone CA9 (transfected with pAnti-IGF IR) and growth of cells of clone ME10 (transfected with vector alone) are compared. Cells were seeded at a density of 2 x 10^3 cells/well and maintained in culture medium containing 10% calf serum. The results shown are representative of an experiment which was repeated three times.

Detailed Description of the Invention

Type I insulin-like growth factor (IGF I) is a ubiquitous peptide that circulates at high levels in serum and is expressed in multiple tissues. It has a broad spectrum of effects including stimulation of embryonic and postnatal growth, cell growth and differentiation *in vitro*, various metabolic effects and participation in tissue regeneration. IGF I also participates in the undesirable proliferation of vascular smooth muscle cells, e.g., associated with post-angioplasty restenosis, restenosis after other vascular injury and with post-cardiac transplant atherosclerosis. The structure of IGF I is known, and the structure and recombinant expression of human IGF I are described in, e.g., U.S. Patent No. 5,324,639 (Brierly et al) and in Gritz and Davies (1983) *J. Gene* 25:179-1[illegible].

The effects of IGF I are mediated via b[illegible]nding to a specific heterotetrameric membrane receptor (IGF IR) that consists of two extracellular α-subunits and two membrane-spanning ß-subunits, which receptor is widely distributed in mammalian tissues. Several studies have suggested that IGF I plays a unique role as an autocrine regulator of cellular function and that activation

of the IGF I/IGF IR loop is required for the entry of cells into S phase in response to a variety of growth stimuli. Ullrich et al (1986) *EMBO J* 5: describes the cDNA coding sequence (see also SEQ ID NO:10) and deduced amino acid sequence of human IGF IR.

SEQ ID NO:10 presents the human IGF IR cDNA sequence and predicted amino acid sequence. The N-termini of the α and the ß subunits are at amino acids 1 and 711, respectively. Potential N-linked glycosylation sites are at amino acids 21-23, 72-74, 105-107, 214-216, 284-286, 397-399, 408-410, 504-506, 577-579, 592-594, 610-612, 726-728, 734-736, 870-872, 883-885 and 933-935. The transmembrane domain is at amino acids 906-929. A potential ATP binding site is at Gly residues at amino acid numbers 976, 978 and 981 and at Lys 1003. The putative precursor processing site is at 707-710, proteolytic cleavage here divides the precursor into the α and ß subunits.

The involvement of IGF I and of the IGF IR in cardiovascular pathology has recently raised interest. *In vitro* data have established that IGF I is a smooth muscle cell mitogen (Pfeifle et al (1982) *Horm. Metab. Res.* 14:409-414; Clemmons, D.R. (1985) *Endocrinology* 117:77-83; Clemmons and Van Wyk (1985) *J. Clin. Invest.* 75:1914-1918) and several reports have documented that vascular smooth muscle cells (VSMC) express IGF I and its receptor *in vitro* and *in vivo* (See, e.g., Jialal et al (1985) *Endocrinology* 117:1222-1229; King et al. (1985) *J. Clin. Invest.* 75:1028-1036; Delafontaine et al (1991) *Hypertension* 18:742-747; Bornfeldt et al (1991) *Diabetologia* 34:307-313). In conditions in which there is excessive VSMC proliferation such as coarctation hypertension (Fath et al (1993) *Circul. Res.* 72:271-277), or after balloon angioplasty (Khorsandi et al (1992) *J. Clin. Invest.* 90:1926-1931), there is an increase in IGF I mRNA expression. Angiotensin II (Ang II), a vascular peptide that is a VSMC mitogen, transcriptionally regulates the IGF I gene in VSMC, and neutralization of extracellular IGF I abolishes Ang II-induced growth. Growth factors such as Ang II, platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) increase IGF I receptors on VSMC, suggesting that upregulation

of IGF I receptors may play a role in growth factor induced mitogenic responses.

In general, the terminology used herein is standard, as understood by those of ordinary skill in the fields of molecular biology, biochemistry, cell biology and medicine. For added clarity, certain terms are defined herein.

As used herein, an antisense oligonucleotide is one which has a nucleotide sequence that is complementary to a particular target nucleic acid molecule, usually a mRNA. In order to effectively inhibit expression of the target mRNA, an antisense is preferably from about 14 nucleotides in length up to the entire target sequence. Oligonucleotides which are chemically synthesized are preferably about 18-22 nucleotides in length. When the antisense molecule is an antisense RNA, i.e., a transcription product within a target cell, preferably the antisense RNA is at least about 100 nucleotides long, and may be as long as 1 Kb or larger. It is generally preferred that the nucleotide sequence of the antisense oligonucleotide is targeted to the region of the mRNA which includes the translation start site.

As used herein, a sense oligonucleotide is one which has a sequence identical to at least a portion of a target nucleic acid molecule, usually a target mRNA. However, a sense oligonucleotide introduced into or expressed in a cell may complex with a naturally occurring target nucleic acid molecule, or which significantly differs in overall nucleotide sequence (by more than about 45%) from a molecule of the same sense polarity.

In the instant context, an ATG-directed oligonucleotide can be sense or antisense relative to a target mRNA. ATG-directed means that the oligonucleotide comprises the ATG or AUG translation start site in a sense oligonucleotide, or its complement CAT (or CAU in RNA) in the antisense oligonucleotide, and the limitations on length and possibly advantageous modifications described herein. An ATG-directed sense of antisense RNA (a less than full length transcript specific for IGF IR or other growth factor receptors) similarly comprises the ATG (or AUG) translation start site or its CAT (or CAU in RNA)

complement, depending on whether it is sense or antisense, and with length limitations as described herein.

In the present context, a mismatch (or missense) antisense oligonucleotide is one which has an antisense nucleotide sequence in which there is significant mismatch with a complementary target nucleic acid molecule.

Where persistence of an oligonucleotide is useful (within a cell, in serum, in cell culture, in a therapeutic composition which has been applied to a target tissue), it is desirable to use an oligonucleotide comprising chemical modifications which contribute to chemical stability, resistance to enzymatic degradation· and the like. Such chemically modified oligonucleotides include, but are not limited to, phosphorothioate (e.g., 2-O-methylphosphorothioate), phosphorodithioate, phosphotriester, methylphosphonate, phosphoramidite, α-anomer and phosphoroselenoate oligonucleotides. The ordinary skilled artisan can prepare such modified oligonucleotides using techniques and equipment well known and readily accessible to the art, and without the expense of undue experimentation (See, e.g., Helene and Toulme (1990) *Biochem. Biophys. Acta.* 1049:99-125 and references cited therein). The skilled artisan knows how to advantageously choose a particular modification for a particular application.

Phosphorothioate (sulfur-protected) oligonucleotides are relatively stable in serum, with about 50% remaining intact after 36 hours incubation, according to Shi et al (1993) *Circulation* 88:1190-1195. This reference describes uptake by and persistence in and inhibition of human smooth muscle cell proliferation by antisense phosphorothioate oligonucleotides directed against c-myc messenger RNA.

A non-naturally occurring recombinant nucleic acid molecule (DNA or RNA) is one which does not occur in nature, and it is thus distinguished from a chromosome, for example. A specific example of a recombinant DNA molecule of the present invention is a vector comprising a portion of an IGF IR sequences operably linked to expression control sequences such that transcriptional expr ssion of at least a portion of the IGF IR sequences is

obtained. Those expression control sequences regulate and promote transcription, and they are not associated with IGF IR coding sequences in nature. Depending on the orientation of the IGF IR sequences relative to the expression control sequences, the transcription product will be sense or antisense, as understood in the art. In a specific embodiment, the transcript is an antisense RNA and is complementary to at least about 100 nucleotides of the native cellular IGF IR mRNA. Alternatively, the expression product is a sense RNA which is shorter than native cellular IGF IR mRNA, does not contain a full-length coding sequence, but which includes the region of the ATG translation start site. Preferably, the sense RNA is significantly shorter than the full length coding sequence, e.g., about 100 nucleotides in length. An additional embodiment is a vector in which a vertebrate IGF IR cDNA sequence with full length coding sequence (e.g. rat) has been inserted, operably linked to expression control sequences.

Vectors may be plasmids or virus vectors capable of being maintained in bacterial cells, yeast cells or in vertebrate, such as mammalian cell culture. These vectors may be designed for maintenance in a single-celled host (bacterium or yeast) and capable only of transient gene expression when introduced into a human, or other animal or capable of stable maintenance with either regulated (temporally and/or tissue-specific) or constitutive gene expression. The art knows how to choose vectors and expression control sequences to achieve a particular desired result from a number of known and readily accessible vectors and expression control sequences known to the art.

For wound and burn healing, bone repair and for collateral vascular development, transient expression is preferred. For antisense RNA expression in treatment and/or prevention of atherosclerosis, post balloon angioplasty restenosis and post transplant atherosclerosis and vascular smooth muscle cell hyperproliferative disorders, transient expression of antisense RNA is preferred. For uses where transient expression of an IGF IR-specific antisense RNA is appropriate, antisense oligonucleotides can be substituted. In applications of

stimulating IGF IR gene expression, such as in the production of leaner meat in, e.g., swine or beef cattle, prolonged expression of a relatively short (at least 14-20 nucleotides, but less than full length) sense ATG-directed IGF IR RNA is preferred.

The cDNA sequence for rat brain cell IGF IR was determined from several overlapping clones isolated as described in Example 1. Nucleotide sequence analysis of the cDNA resulted in deduction of the primary protein sequence of the rat IGF IR precursor (SEQ ID NOS: 8 and 9). The cDNA analyzed contains an opening reading frame of 4113 nucleotides encoding a 1370 amino acid IGF IR precursor, with 45 bp of 5' untranslated and 538 bp of 3' untranslated flanking sequences. Based on the structure of the human IGF IR (Ullrich et al. (1986) supra), the first 30 encoded amino acids likely represent a signal peptide (amino acids -30 to -1) with the putative start site of the mature α subunit being glutamate at position 1 (SEQ ID NO:9). Four arginine residues representing the putative cleavage site of the $\alpha\beta$ proreceptor precede the putative start site of the ß subunit at Asp 712. The 17 amino acid hydrophobic sequence between residues 908 and 924 is likely a transmembrane domain. Features consistent with kinase activity are located between amino acids 974 and 1230, including the tyrosine kinase class II signature pattern from Asp 1130 to Arg 1138 and a potential ATP-binding region from Leu 976 to Val 984. Lammers et al. (1989) *EMBO J.* 8:1369-1375 describes a recombinant vector for the expression of human IGF IR.

Analysis of the full-length rat IGF IR cDNA sequence (SEQ ID NO:8) revealed a high overall degree of similarity to the human sequence (Ullrich et al. (1986) supra). Within the protein coding sequence there is approximately 89% sequence identity at the nucleotide level and about 98% sequence identity at the amino acid level. The deduced amino acid sequence is given in SEQ ID NO:9. The predicted rat α-chain comprises 707 amino acids (compared with 706 for the human) with an 89% and 98% similarity to the human IGF IR α-chain at the nucleotide and amino acid levels, respectively. There are 32 cystein residues within the α-chain, including 24 cysteine residu s in a cysteine-rich region

extending from Cys 148 to Cys 302. These are perfectly conserved between the rat and human receptors, consistent with the proposed important function of disulfide bridging in assembly of the α_2-$ß_2$ mature receptor. A putative cleavage site formed by four arginines (contrasting with the Arg Lys Arg Arg sequence of the human receptor) after residue 707 precedes the putative start site of the ß subunit at Asp. Like the α-chain, the ß-chain is highly conserved between rat and human with 90% and 98% similarity at nucleotide and amino acid levels. A 17 amino acid hydrophobic sequence (residues 908 to 924) likely represents the transmembrane domain, and differs from the corresponding human sequence by the substitution of an isoleucine for valine at position 915. The highly conserved tyrosine kinase domain contains a protein kinase ATP binding signature (Leu 976 to Val 1084), a tyrosine kinase specific active-site signature (Phe 1102 to Val 1114), and a receptor tyrosine kinase class II signature (Asp 1130 to Arg 1138). These sites are perfectly conserved between rat and human, as are the positions of cysteines within the rat and human ß-chains. Like the human receptor, there are 17 potential N-glycosylation sites in the rat receptor. In contrast to the coding sequence, the 3'-untranslated region of the rat IGF IR has a lower (72%) degree of similarity to the human sequence.

As shown in Figure 1, incubation of VSMC with increasing concentrations of Antisense (AS) oligonucleotides (ODN) (SEQ ID NO:1) for 48 hr in serum-free medium decreased [^{3}H]-thymidine incorporation (69 ± 7.6% decrease with a concentration of 10 μM, as compared with serum-free medium alone). In addition, the mitogenic response to 10% calf serum was inhibited (56% decrease with a concentration of 10 μM, as compared with 10% calf serum alone). There was no significant change in either basal or serum-ind d DNA synthesis rates in cells exposed to mismatch antisense) oligonucleotides (SEQ ID NO:3). Surprisingly, incubatic.. of VSMC in serum-free medium for 48 hr with sense (S) oligonucleotide (SEQ ID NO:2) significantly increased [^{3}H]-thymidine incorporation - 145% increase above serum-fee medium alone, with a concentration of 10 μM S oligonucleotides.

Furthermore, the mitogenic response to 10% calf serum was maintained in cells pre-exposed to sense ATG-directed oligonucleotide. Thus the incorporation of [^{3}H]-thymidine into cells exposed to 10 μM S ODN (SEQ ID NO:2) for 48 hr and then to 10% calf serum was 61% greater than that of cells exposed to serum-free medium alone for 48 hr and subsequently to 10% calf serum. In order to establish correlations between DNA labeling indices and cell proliferation, cell counts were performed. Treatment of VSMC with 10 μM AS oligonucleotide (SEQ ID NO:1) for 96 hr in the presence of 10% calf serum reduced cell number by 58% compared with cells maintained in 10% calf serum alone (Fig. 2). M ATG-directed oligonucleotides (SEQ ID NO:3) had no effect on cell proliferation, whereas ATG-directed S oligonucleotide (SEQ ID NO:2) increased cell number (30% increase with 10 μM S ODN over 10% calf serum alone).

To assess the effects of oligonucleotides on IGF I-induced growth (mitogenic) response, cells were incubated in 10% calf serum ± oligonucleotides (ODNs) for 48 hr and then exposed to serum-free medium in the presence or absence of increasing concentrations of IGF I (Fig. 3). IGF I caused a dose-dependent increase in [^{3}H]-thymidine incorporation that was markedly blunted by pre-incubation of cells with ATG-directed AS ODNs. Thus, [^{3}H]-thymidine incorporation stimulated by 20 ng/ml IGF I was reduced by 62% in AS-treated cells, compared with control. Exposure of cells to 5 μM S ODN increased [^{3}H]-thymidine incorporation basally (187% above control) and in response to IGF I (67% increase above control following incubation with 20 ng/ml IGF I).

A neutralizing antibody specific for IGF I inhibits ang II-induced DNA synthesis in VSMC (Delafontaine et al. (1991) *J. Biol. Chem.* 268:16866-16870). These findings suggested that activation of the IGF IR was required for Ang II-induced growth (mitogenic) responses in VSMC. We therefore assessed the effects of ODNs on ang II-induced [^{3}H]-thymidine incorporation. As shown in Figure 4, Ang II caused a dose-dependent increase in DNA synthesis that was unaltered by pre-exposure of cells to ATG-directed M ODN (SEQ ID NO:3) but was markedly reduced following

pre-exposure to ATG-directed AS ODN (SEQ ID NO:1). Thus, there was a 75% reduction in [^{3}H]-thymidine incorporation in response to 100 nM ang II in AS-treated cells, compared with control. S-treated cells (SEQ ID NO:2) had a marked increase in [^{3}H]-thymidine incorporation basally (297% above control). Ang II had no additive mitogenic effect on S-treated cells.

To establish correlations between the observed effects of ODNs on growth and IGF IR binding parameters, radioligand binding experiments were performed. Exposure of VSMC to 5 μM ATG-directed AS ODN (SEQ ID NO:1) for 48 hr in serum-free medium reduced IGF IR number by 56%, compared with control (Fig. 5A). M ODN (SEQ ID NO:3) had no effect on IGF I binding capacity, while S ODN (5 μM) (SEQ ID NO:2) increased IGF IR number by 39%. Exposure of VSMC to ODNs in the presence of 10% calf serum produced similar results (Fig. 5B). Thus, 5 μM AS ODN (SEQ ID NO:1) reduced IGF IR by 57% and 5 μM S ODN (SEQ ID NO:2) increased IGF IR by 69%, compared with 10% calf serum alone. There was no effect of IGF IR ODNs on IGF I binding-affinity. To further confirm the specificity of these findings, [^{125}I-]sar^{11}-ile^{8}ang II binding studies were performed on cells exposed to IGF IR ODNs. AS, S, and M ODNs (SEQ ID NOS:1, 2 and 3, respectively) had no effect on ang II receptor number (Fig. 5C) or affinity.

To determine whether the observed effects of S ODN (SEQ ID NO:2) on VSMC IGF IR number and growth responses were site-specific, experiments were conducted using AS and S oligomers targeting a sequence starting at 109 nucleotides downstream of the ATG. As shown in Fig. 6A, AS ODNs of SEQ ID NO:4 produced the expected reduction in [^{3}H]-thymidine incorporation, basally and in response to 10% calf serum. However, in marked contrast to results obtained using S ODNs targeting the ATG site (i.e., SEQ ID NO: 2), S ODNs targeting the sequence about 100 bases 3' to the ATG site (SEQ ID NO:5) did not significantly alter DNA synthesis, either basally or in response to serum. Furthermore, radioligand binding studies establish d that these ODNs had the corresponding effects on IGF IR number. Thus, exposure of VSMC to 5 μM SEQ ID NO:4 AS ODN in the presence of serum-free medium or 10% calf serum reduced IGF IR number by 28% and 38%

respectively, while there was no significant effect of SEQ ID NO:5 S ODNs on IGF IR number (Fig.6B).

To determine whether effects of ODNs on IGF IR number correlated with changes in steady-state levels of IGF IR mRNA, solution hybridization/RNase protection assays were performed (Fig. 7). AS oligonucleotides complementary to IGF IR mRNA decreased IGF IR mRNA levels (~40% decreases with 5 μM ODNs directed at ATG and non-ATG sites (SEQ ID NOS:1 and 4, respectively). S ODNs (SEQ ID NO:2) targeting the ATG site did not significantly increase IGF IR mRNA levels, while S ODNs (SEQ ID NO:5) targeting the sequence about 100 bases 3' to the ATG site did not significantly affect IGF IR mRNA levels.

To further address the function of the IGF IR in VSMC growth, an antisense IGF IR construct was stably transfected into primary rat aortic smooth muscle cells (RASM). Our results show that VSMC expressing this antisense transcript have a decrease in endogenous IGF IR mRNA levels, particularly at confluence and post-confluence. The reduction in IGF IR mRNA levels in post-confluent cells of ~57% correlates well with the 51% decrease in surface IGF IR binding sites. DNA synthesis in antisense-expressing cells was decreased by 55%, indicating that a reduction in IGF IR number/cell inhibited VSMC growth. Furthermore, antisense-expressing cells had a 59% decrease in IGF I-stimulated DNA analysis. These findings were substantiated by growth curves which showed a marked reduction in the proliferation of CA9 cells maintained in 10% serum. Interestingly, there was only a small decrease in IGF IR mRNA levels in antisense-expressing cells at preconfluence, correlating with a 35% reduction in IGF IR number. The lesser reduction in IGF IR mRNA in preconfluent cells may relate to the fact that preconfluent control cells have approximately 1.5-fold higher basal IGF IR mRNA levels than post-confluent cells. The greater reduction in IGF IR at confluency in antisense cells correlates with a marked reduction in cellular growth rates. Because serum contains a variety of growth factors, these results suggest that the IGF IR participates in growth responses to multiple agonists.

In order to detect antisense IGF IR mRNA, northern hybridization analysis was performed using a ^{32}P-labeled sense riboprobe generated using T7 polymerase transcription of a partial IGF IR sequence (nucleotides +560 to +680). CA9 cells (which express antisense RNA from a vector, see Example 3 and 5), at both preconfluence and post-confluence expressed abundant amounts of the 1.0 kb antisense transcript, while this transcript was undetectable in control clone ME10 (containing vector without IGF IR-related insert). To determine the effect of antisense IGF IR mRNA on endogenous IGF IR mRNA levels, solution hybridization/RNase protection assays were performed using an antisense IGF IR riboprobe, co-hybridized with an antisense GAPDH riboprobe as an internal control. There was a small reduction in IGF IR mRNA levels in preconfluent cells and marked reduction in post-confluent cells. Densitometric analysis of IGF IR mRNA levels (corrected for the GAPDH signal) indicated that IGF IR antisense-expressing CA9 cells, compared with ME10 cells, had a reduction in IGF IR mRNA of 18±4.5% (mean ± SE,n=2) at preconfluence, and of 57±4.8% (mean ± SE, n=3) at post-confluence.

In order to assess the effect of antisense IGF IR cDNA transcription on IGF I binding, ^{125}I-IGF I radioligand binding studies were performed with CA9 cells and control ME8 and ME10 cells, all at preconfluence and at confluence/up to 2 days post-confluence. Results from scatchard analysis of displacement curves indicated that there was a 35% reduction in IGF IR number/cell in preconfluent CA9 cells, as compared with preconfluent control ME8/ME10 cells (CA9, 44 ± 1.0 fmol/10^5 cells; ME8 and ME10, 68 ± 2 fmol/10^5 cells, n=2 There was a 51% reduction in IGF IR ber in confluen .9 cells, as compared with confluent or ME1 ells. There was no significant different in IR bind g-affi y between CA9 cells (K_d = 5.6 ± 1.0 nM, n = 10) and ME8/ME10 ells (K_d = 6.7 ± 1.5 nM, n = 10), either at preconfluency or confluency.

To determine whether the reduction in IGF IR density on CA9 cells altered DNA synthesis, [^{3}H]thymidine incorporation was measured in CA9 cells and in control ME10 cells. There was a 55%

reduction in [^{3}H]thymidine uptake in CA9 cells as compared with ME10 cells. To determine whether the mitogenic response to IGF I was altered by reduction in IGR IR density, DNA synthesis was measured in confluent cells exposed to IGF I (10 ng/ml, 24 hr.). There was a 59% reduction in IGF I-stimulated [^{3}H]thymidine incorporation in CA9 cells (585 ± 198 dpm/10^4 cells, n = 3) as compared with ME8/ME10 cells (1428 ± 92 dpm/10^4 cells, n = 2).

To further characterize the effect of IGF IR antisense targeting on cell proliferation, growth curves were performed using CA9 and ME10 cells maintained in the presence of 10% calf serum. There was a marked inhibition of the proliferative response of CA9 cells. The antiproliferative effect became evident at day 7, as the cells reached confluence. The mean of results from three independent experiments indicated that there was a 60 ± 3.4% reduction in cell number at day 7 after plating in CA9 cells, as compared with ME10 cells.

Anti-IGF I antiserum has been shown to inhibit PDGF-mediated growth of VSMC (Clemmons and Van Wyk (1985) supra). Delafontaine and Lou (1993) supra showed that Ang II transcriptionally regulated IGF I expression in VSMC and that anti-IGF I antibody inhibits Ang II induced DNA synthesis. In neuroepithelial cells FGF-mediated proliferation is dependent on IGF I (Drago et al. (1991) *Proc. Nat'l Acad. Sci. USA* 88:2199-2203).

There is some experimental suggestion of the importance of IGF IR number/cell in cellular growth responses. Antisense targeting of the IGF I/IGF IR system has provided evidence for its role in normal organ growth (Wada et al. (1993) *Proc. Nat'l. Acad. Sci. USA* 90:10360-10364) and in tumorigenesis (Trojan et al. (1992) *Proc. Nat'l. Acad. Sci. USA* 89:4874-4878). Furthermore, overexpression of the IGF IR has been shown to induce transformation of NIH/3T3 cells *in vitro*, allowing these cells to form tumors in nude mice (Kaleko et al. (1990) *Mol. Cell. Biol.* 10:464-473). The results presented herein, taken with what was known, indicate that IGF IR plays a crucial role in VSMC proliferative responses and provides the basis for antisense methods to down-regulate IGF IR gene expression to

retard VSMC growth and proliferate where undesirable, e.g., in treatments to prevent post angioplasty restenosis, restenosis after vascular injury or post-heart-transplant atherosclerosis in the coronary arteries and vascular complications of hypertension including proliferative retinopathy, cerebrovascular disease and hypertensive nephropathy. And, on the contrary, ATG-directed sense oligonucleotides and methods using same allow for stimulation of cell growth and proliferation where desirable. Such situations where increased IGF IR number/cell is desirable include healing of broken bones, burns and other wounds, nerve regeneration after injury, and angiogenesis in tissue affected by peripheral vascular disease or by myocardial infarction.

The foregoing results clearly demonstrate that IGF IR density on vascular smooth muscle cells is an important determinant of their growth responses to serum and to angiotensin II. Thus, targeting of the IGF IR mRNA with antisense ODNs complementary thereto, as exemplified with either a sequence spanning the translation initiation codon or the sequence beginning about 109 bp downstream thereof, reduces IGF IR mRNA levels and IGF IR number without altering IGF IR binding-affinity (K_d). This inhibition of IGF-I Receptor expression results in a decrease in basal [^{3}H]-thymidine incorporation as well as in the mitogenic response to the addition of 10% serum. The antiproliferative effect of the antisense oligonucleotides is reflected in the reduction of cell counts after 96 hr incubation of VSMC with increasing doses of AS ODN in the presence of 10% calf serum. Because serum contains a variety of growth factors, DNA synthesis rates in response to IGF I were measured; [^{3}H]-thymidine incorporation in response to IGF I was inhibited in a dose-dependent manner by antisense oligonucleotides. The mitogenic response to ang II was also inhibited by antisense oligonucleotides complementary to IGF IR mRNA, confirming the crucial role of the autocrine IGF I ligand-receptor system in ang II-induced growth of VSMC. It was previously demonstrated that an anti-IGF I antibody inhibits ang II-induced mitogenesis in VSMC (Delafontaine et al. (1993) supra). The effect of IGF IR antisense oligonucleotides on ang II mitogenesis was specific to

IGF IR gene expression, in that ang II binding capacity was not altered.

In contrast to findings with antisense oligonucleotides, an ATG-directed mismatched antisense oligonucleotide (M, SEQ ID NO:3) (based on the antisense sequence of SEQ ID NO:1 but with a 9 of 20 bp mismatch) had no effect on IGF I receptor number, [^{3}H]-thymidine incorporation (basally or in response to serum), cell proliferation and the mitogenic response to ang II. These data confirm the specificity of the molecular effects of IGF IR antisense oligonucleotides.

Unexpectedly, exposure of VSMC to a sense oligonucleotide corresponding to sequence spanning the initiation codon of the IGF IR (e.g., S, SEQ ID NO:2) resulted in an increase in IGF IR number per cell, associated with an increase in basal [^{3}H]-thymidine incorporation (in serum-free medium) as well as in the presence of 10% serum. This was reflected in an increase in cell proliferation. The increase in IGF IR density induced by S ODN of SEQ ID NO:2 was associated with an increase in the mitogenic response to IGF I. Interestingly, however, ang II had no additive stimulatory effect on [^{3}H]-thymidine incorporation following pre-incubation of cells with S ODN (SEQ ID NO:2). This suggests that at a certain level of activation of the IGF IR, angiotensin II does not further stimulate mitogenesis.

Molecular mechanisms responsible for ATG-directed S oligonucleotide-mediated increases in IGF IR number have not yet been elucidated. The increase is associated with an increase in IGF IR mRNA levels and is sequence-specific. Thus, although an AS ODN targeting a sequence at bp + 109 (relative to ATG) (SEQ ID NO:4) reduced IGF receptor number and inhibited VSMC growth, the corresponding sense oligonucleotide targeting this site (SEQ ID NO:5) had no effect on either IGF IR number of VSMC growth. Without wishing to be bound by any particular theory, one potential explanation for S ODN-mediated upregulation of IGF IR is the presence of an endogenous IGF IR antisense mRNA species in VSMC, which is effectively "neutralized" by the administered sense oligonucl otide. However, reports of naturally occurring antisense RNAs in eukaryotic systems are rare (Inouye, M. (1988)

Gene 72:25-34; Colman, A. (1990) *J. Cell. Science* 97:399-409; Helene et al. (1990) *Biochem. Biophys. Acta.* 1049:99-125).

Alternatively, and again without wishing to be bound by any particular theory, the S mRNA may bind to its complementary DNA sequence through Watson-Crick bonding or through triple helix formation and increase transcription by facilitating the opening of the DNA helix. Indeed, local openings of the double helix have been shown to correlate with DNA template activity, and clearly, epigenetic RNA molecules are capable of stabilizing these openings (Reiss et al. (1991) *Cancer Research* 51:5997-6000. A third non-binding potential explanation is that the ATG-directed sense oligonucleotide may bind with a protein factor to effect positive regulation of IGF IR gene expression.

These data indicating a key regulatory role of the IGR IR in VSMC growth are consistent with other observations in fibroblasts. In BALB/c3T3 fibroblasts, Epidermal Growth Factor (EGF) upregulates IGF I expression; and secretion and targeting of the IGF IR through use of antisense oligonucleotides inhibits EGF-induced growth (Pietrzkowski et al. (1992) *Mol. Cell. Biol.* 12:3883-3889). Furthermore, in BALB/c3T3 cells overexpressing IGF I and IGF IR, IGF I-mediated growth occurs independently of the EGF and PDGF receptors (Pietrzkowski et al. (1992) *Cell Growth Differ.* 3:199-205). Constitutive expression of c-myb in 3T3 cells has been shown to upregulate IGF I and IGF IR expression, thereby abrogating the requirement of these cells for exogenous IGF I and suggesting that IGF IR activation may be important mechanically in the effect of c-myb on cell proliferation (Reiss et al. (1991) supra; Travali et al. (1991) *Mol. Cell. Biol.* 11:731-736).

Moreover, in SV40 T antigen-transformed BALB/c3T3 cells, antisense targeting of the IGF I receptor has been shown to inhibit growth (Porcu et al. (1992) *Mol. Cell. Biol.* 12:5069-5077). The results provided herein are strong evidence that IGF IR density is an important factor in mediating IGF I and serum-induced growth responses in vascular smooth muscle cells. Thus, even in the presence of high concentrations of IGF I, the mitogenic response of antisense-treated cells is markedly

blunted. The data suggest that IGF I, acting through its tyrosine-kinase receptor, may serve as an important co-factor or intermediary in the growth response to a variety of agonists. This is consistent with its known effects at the G_1/S phase of the cell cycle (Stiles et al. (1979) *Proc. Natl. Acad. Sci USA* 76:1279-1283).

Manipulation of IGF IR density on vascular smooth muscle cells markedly alters the growth responses of these cells to IGF I, ang II and serum. Receptor availability has marked effects on ligand responsiveness. This ligand-receptor system is crucial for the control of VSMC growth *in vitro* and *in vivo*. Furthermore; the surprising observation that a sense oligonucleotide targeting of the ATG site of the IGF IR message upregulates IGF IR identifies a novel use for synthetic oligonucleotides in the regulation of gene expression.

As demonstrated herein, the density of IGF IR can be down-regulated via antisense oligonucleotides or antisense RNAs complementary to at least a portion of the IGF IR mRNA (≥14 contiguous nucleotides for oligonucleotides, at least 100 nucleotides for intracellularly transcribed antisense RNAs). Antisense oligonucleotides and antisense RNAs inhibit vascular smooth muscle cells *in vitro* and have the same effect *in vivo*.

Generally, when oligonucleotides are used, they are in a modified form which increases persistence in cells and/or in the extracellular milieu. An exemplified modified modification is phosphorothioate oligonucleotides. ATG-directed sense oligonucleotides (e.g., phosphorothioate oligonucleotides) specific for IGF IR mRNA can be delivered when the proliferation of vascular smooth muscle cells, or when collateral blood vessel development is desired. For delivery of oligonucleotides or recombinant vectors to the inner surfaces of an artery, a catheter can be used or a porous balloon generally similar to that used in balloon angioplasty can be used (See, e.g., Shi et al. (1994) *Circulation* 90:944-951; Guzman et al. (1993) *Circulation* 88:2838-2848; Ohno et al (1994) *Science* 265:781-784).

For wound, burn and bone healing applications or where collateral blood vessel development, angiogenesis or nerve

regeneration is desired, ATG-directed, IGF IR-specific oligonucleotides, preferably modified for resistance to degradation (e.g., in phosphorothioate form), are formulated into pharmaceutical compositions.

For delivery to wounded or burned tissue or damaged bone, pharmaceutical preparations of IGF IR-specific, ATG-directed sense oligonucleotides, preferably a modified oligonucleotide such as a phosphorothioate oligonucleotide can be formulated, in a therapeutically effective amount, with a suitable carrier for topical application or other local application such as introduction via catheter. An adenovirus vector capable of expressing an IGF IR-specific, ATG-directed sense RNA can also be formulated into a healing-promoting preparation. Preferably such a vector is replication-deficient.

Where inhibition of IGF IR receptor gene expression is desired, an IGF IR-specific antisense oligonucleotide (such as a phosphorothioate oligonucleotide) or an antisense-expressing recombinant vector can be incorporated. In a situation where angiogenesis is advantageously inhibited an IGF IR-specific antisense-expressing recombinant vector or oligonucleotide preparation can be directed to a target tissue such as a solid tumor using a means known to the art. One example is the delivery to prostate tumor or other tumor bearing androgen receptors using the methods and compositions disclosed in USSN 08/283,238 filed July 29, 1994, incorporated by reference herein in its entirety.

Suitable excipients for therapeutic/pharmaceutical compositions are described in, e.g., Remington's Pharmaceutical Sciences, by E.W. Martin. Other components such as stabilizers including chelating agents, e.g., EDTA, sugars, sugar alcohols, antioxidants and nonionic surfactants such as polyethylene glycol or block co-polymers of polyethylene and polypropylene glycol may be added.

Therapeutic oligonucleotide (or vector) compositions of the present invention contain a therapeutically effective dose of the active ingredient in a pharmaceutically acceptable carrier. The dose, carrier, route and schedule of administration of choice

depend among other factors, upon each other, the condition to be treated and the patient's species. These factors are readily determined and monitored by the treating physician or veterinarian during the course of therapy.

All references cited herein are hereby incorporated by reference in their entirety.

Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) *Meth Enzymol.* 68; Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold spring Harbor Laboratory, Cold Spring Harbor, New York, Old Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

The foregoing discussion and the following examples illustrate but are not intended to limit the invention. The skilled artisan will understand that alternative methods and compositions may be used to implement the invention.

EXAMPLES

Example 1 Cloning of IGF I Receptor cDNA

Total RNA was isolated from rat aortic smooth muscle cells (RASM) and following first strand cDNA synthesis a 1.1 kb cDNA probe was generated using Polymerase Chain Reaction, PCR. Primers D1 (5'-CCCCAAATAAAAGGAATG-3) (SEQ ID NO:6) and U1 (5'-AGCAGATTGCCCTTC-3')(SEQ ID NO:7) were based on the sequence of Werner et al. (1989) *Proc. Nat'l Acad. Sci. USA* 86: 7451-7455. The PCR product was directly cloned into the pCRII vector (Invitrogen Inc., San Diego, CA), and the resulting clone (p118) was sequenced and used to screen a λgt10 cDNA library from adult male Sprague-Dawley rat brain (Clontech, Palo Alto, CA). 1.2 x 10^6 clones were screened, and 30 of 80 positive clones were analyzed by PCR using a λgt10 arm universal primer and an IGF IR internal primer to determine insert size. After two additional cycles of screening, clones containing longer inserts were isolated, purified and the library was rescreened using these clones and a 3' specific probe (from 2887-2906 bases downstream of the IGF IR translation start site). EcoRI inserts of purified phage clones were subcloned into pGEM3 (Promega Corp., Madison, WI) and sequenced using the sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, OH). Ambiguities were resolved by resequencing using a modified 40% formamide gel (J.T. Baker, Inc., Phillipsburg, NJ). DNA sequence data was assembled from nine overlapping clones and analyzed using the Genetics Computer Group program (Madison, WI). The deduced amino acid sequence data was analyzed using PC Gene (IntelliGenetics, Inc., Mountain View, CA). A full-length rat cDNA clone for IGF IR was also isolated.

Example 2 Oligonucleotide Synthesis

Phosphorothioate 20-mer oligonucleotides (ODNs) synthesized using an Applied Biosystems instrument (Applied Biosystems, Foster City, CA) by the Microchemical Facility, Emory University, HPLC purified, resuspended in TE (10 mM Tris, 1 mM EDTA; pH 8.0), and quantified by spectrophotometry. Antisense (AS), sense (S) and mismatch (M) ODNs targeting a sequence starting 2 nucleotides

5' to (upstream of) the ATG site of rat IGF IR cDNA were respectively; AS, 5'-TCCGGAGCCAGACTTCATTC-3' (SEQ ID NO:1), S, 5'-GAATGAAGTCTGGCTCCGGA-3' (SEQ ID NO:2); M, 5'-AGCGGTCCCACTCTTGTTTG-3' (SEQ ID NO:3). ODNs targeting a sequence starting 109 bases downstream of the ATG were AS, 5'-CAGCTGCTGATAGTCGTTGC (SEQ ID NO:4) and S, 5'-GCAACGACTATCAGCAGCTG-3' (SEQ ID NO:5). Oligonucleotides were filter-sterilized and used at a concentration of 0.1-10 μM.

Example 3 Construction of Antisense Rat IGF IR Vector.

A diagrammatic representation of the vector assembly is shown in Figure 8. Briefly, the rat IGF IR cDNA clone p118 was digested by XhoI and KpnI and the 0.8 kb restriction fragment (from nucleotide +277 to +1086 relative to ATG) was ligated in an antisense orientation into pCEP4 (Invitrogen). (See also SEQ ID NO:8.) Transcription of the antisense IGF IR cDNA is under control of the cytomegalovirus immediate early gene enhancer/promoter, and yields a 1.0 kb antisense transcript (including linker and polyadenylated tail). This vector contains two Epstein Barr virus (EBV) derived genes namely, the EBV ori-P (origin of replication) and the EBV-nuclear antigen 1, which allow episomal replication. The SV40 poly(A) tail provides a termination signal for transcription, and a hygromycin resistance gene allows selection of transfectants.

Example 4 Cell Culture

Vascular smooth muscle cells (VSMC) were isolated from rat thoracic aorta by enzymatic dissociation as described by Gunther et al. (1982) *J. Cell. Biol.* 92:289-298. They were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (CS), 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, and passaged twice a week by harvesting with trypsin-versene and seeding at a 1:8 ratio in 75 cm^2 flasks.

For oligonucleotide experiments, cells between passage levels 5 and 15 were seeded into 100-mm, 24-well or 48-well polystyrene cluster culture dishes. For certain experiments cells were quiesced by exposure to defined serum-free medium

(SFM) containing DMEM and Ham's F12 (1:1) supplemented with transferrin (5 μg/ml), insulin (5 x 10^{-7} M), ascorbate (0.2 mM), glutamine and antibiotics.

Example 5 Cell Transfection and Selection

Vascular smooth muscle cells were isolated an cultured as described in Example 4 hereinabove. For transfections, ~10% confluent (passage number five) cells in 100 mm dishes were incubated in the presence of 10 μg of calcium phosphate precipitate of carrier DNA (pRSV-CAT, Invitrogen) and the plasmid pAnti-IGF IR in a 1:2 ratio for six hours as described by Chen and Okayama (1988) *Bio Techniques* 6:632-638. Cells were grown in hygromycin-free medium for 72 hours and then selected in the presence of hygromycin (Sigma) at a concentration of 100 μg/ml. This concentration of antibiotic was determined to effectively kill VSMC. Selection medium was replaced every three days. After six weeks, hygromycin resistant colonies were isolated, cloned into 24 well plates and subsequently expanded into 12 and 6 well tissue culture plates.

Eleven clones stably transfected with pAnti-IGF IR and 25 clones transfected with vector alone were selected by hygromycin resistance and amplified. The presence of the vector sequence in transfected cells was confirmed by Southern blotting, and northern hybridization analysis was used to screen for presence of antisense IGF IR mRNA. Two clones transfected with pAnti-IGF IR exhibited high level expression of the 1 kb antisense IGF IR mRNA, and of these, clone CA9 was selected for further study. Two clones transfected with vector alone (ME8 and ME10) were selected as controls for further characterization.

Example 6 Measurement of DNA synthesis

To measure effects of ODNs on the growth response to 10% serum, VSMC were grown to 80% confluence in 48-well plates, serum-deprived for 48 hr in the presence or absence of ODNs (0.1-10 μM) and then exposed to fresh SFM in the absence of ODNs, or to 10% serum, for 24 hr. For cells transfected to transcribe oligonucleotides, 1 μCi/ml [^{3}H]-thymidine was included during the

last 24 hr. Cells were then washed 3x with ice-cold phosphate buffered saline, incubated on ice for 15 min with 10% trichloroacetic acid (TCA), filtered with two washes in ice-cold 95% ethanol, air dried, and then insoluble radioactivity was extracted with 0.4 N NaOH and counted by liquid scintillation spectrophotometry after neutralization. All experiments were performed in triplicate.

In order to measure the effects of ODNs on the mitogenic response to IGF I, VSMC were grown to 50% confluence and then incubated in DMEM with 10% CS alone, or in the presence of 5 μM AS or 5 μM S ODN for 48 hr. Cells were then washed in serum-free medium (SFM) and incubated in SFM ± IGF I (1-100 ng/ml) for 24 hr. 1 μCi/ml [^{3}H]-thymidine was present during the latter 24 hr., and TCA-precipitable counts were determined as described above.

To measure the effects of ODNs on the mitogenic response to ang II, VSMC were exposed to AS or S ODNs (5 μM) for 48 hr in the presence of 10% CS. Cells were then washed in SFM and exposed to SFM ± ang II (1-1000 nM) for 24 hr. At 24 hr cells were pulsed with [^{3}H]-thymidine (1 μCi/ml) for 12 hr in the continued presence of ang II. TCA-precipitable counts were then determined as described above.

Example 7 Growth Assay

VSMC were grown to 50% confluence in 48-well plates and then exposed to SFM alone, 10% CS alone or 10% CS and increasing concentrations (0.1-10 μM) of AS, S, or M ODNs. Medium and ODNs were replaced at 48 hr. At 96 hr cells were trypsinized and counted. To analyze growth curves on cells with transcribed antisense IGF IR oligonucleotides, transfected cells were split into 24-well culture plates at a density of 2,000 cells per well, and grown in the presence of 10% calf serum and 100 μg/ml hygromycin. Cells in duplicate wells were washed with PBS, harvested with PBS/20 mM EDTA and counted manually. Culture medium replacement and cell counts were performed every two days.

Example 8 Binding Assays

To determine the effect of ODNs on IGF IR number per cell and binding-affinity, VSMC were grown to 80% confluence in 24-well plates, and then exposed to SFM alone or in the presence of AS, S, or M ODNs (5 μM) for 48 hr. For some experiments cells were grown to 50% confluence and then exposed to 10% CS alone or in the presence of AS or S ODNs (1,5 μM) for 48 hr. Cells were washed in PBS and incubated at 37°C for 60 min. in binding buffer (20 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.25% bovine serum albumin, pH 7.4) to allow dissociation of cell-bound IGF I. Cells were then rewashed and then incubated in the presence of 10^{-10} M ^{125}I-IGF I and increasing (0-10^{-7} M) concentrations of unlabeled IGF I for 90 min at room temperature. Cells were washed in ice-cold binding-buffer and solubilized in 2N NaOH before counting using an automated gamma counter with about 80% efficiency. All assays were performed in duplicate for each experimental point. Data were analyzed (scatchard analysis) using the LIGAND program. For measurement of Ang II binding, cells were incubated for 48 hr in 10% CS alone or in the presence of ATG-directed antisense (AS), sense (S) or missense (M) oligonucleotides (5 μM). Binding studies were performed essentially as described above, except 10^{-10} M [^{125}I-Sar^1-Ile^8]-ang II and 0-10^{-5} M unlabelled ang II were used for displacement curves.

For studies of the effects of transcribed IGF IR oligonucleotides, confluent and up to two days post-confluent 24-well culture plates of VSMC transfected with vector alone (ME8, ME10) or with pAnti-IGF IR (C9) were assayed and the data were analyzed as for the oligonucleotide-treated cells.

Example 9 Solution hybridization/RNase Protection Assay

For determination of IGF II mRNA levels a 203 bp EcoRI and KpnI rat IGF IR cDNA fragment was ligated into pGEM3 (Promega Corp., Madison, WI). The subclone p26K was linearized with EcoRI to allow generation of antisense RNA probe using SP6 RNA polymerase. Solution hybridization assays were performed by hybridizing 30 μg of total RNA with 5 x 10^5 cpm [^{32}P]-UTP labelled

AS IGF IR riboprobe and co-hybridizing with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe as previously described (Delafontaine et al. (1991) *Hypertension* 19:693-699; Delafontaine et al. (1992) *Hypertension* 18:742-747; Delafontaine and Lou (1993) *J. Biol. Chem.* 286:16866-16870). In brief, 30 μg of total RNA was hybridized with 5 X 10^5 cpm of [^{32}P]-UTP labeled antisense IGF IR riboprobe and co-hybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe (Fort et al. (1985) *Nucl. Acids Res.* 13:1431-1442) in a solution containing 80% deionized formamide, 40 mM PIPES, pH 6.4, 0.4 M NaCl, 1 mM EDTA. After hybridization at 42°C and RNase digestion using 40 μg/ml RNase and 100 units/ml RNase T1, samples were proteinase K treated, phenol extracted, and analyzed by 6% polyacrylamide/8 M urea denaturing gel electrophoresis. Full-length IGF IR probe is 251 bp, and protected fragment is 195 bp. The GAPDH protected fragment is 156 bp. Autoradiography was performed with 1-3 days exposure, and protected bands were quantitated by two-dimensional laser densitometry.

Example 10 Northern Hybridization Analysis

Transfected VSMC were studied at preconfluence (~80% confluent) and at 2 days post-confluence. Total RNA was prepared from stably transfected VSMC using the TRI-reagent method (Molecular Research Center, Inc., Cincinnati, OH), subjected to 1.2% agarose-formaldehyde gel electrophoresis, and transferred to a nylon membrane (Genescreen Plus, New England Nuclear, Boston, MA). The filter was stained with methylene blue to monitor RNA loading and RNA integrity. A ^{32}P-labeled rat IGF IR sense RNA probe (120 bp, nucleotides +560 to +680) was generated by T7 polymerase transcription of the rat IGR IR cDNA clone p522, after linearizing with PvuII. Hybridization conditions were as recommended by Promega (Madison, WI) i.e., 7% PEG 8000, 7% SDS, 50% formamide, 0.25 M NaCl, 0.25 M $NaPO_4$, pH 7.2, 100 μg/ml herring sperm DNA and 100 μg/ml y ast tRNA for 24 hr at 60°C. Filters were washed at room temperature in 2 x SSC and then at high stringency (0.1 x SSC, 30 min at 65°C) prior to autoradiography.

Example 11 Vectors for Local Synthesis of IGF IR-Regulating Oligonucleotides

Where up-regulation of IGF IR gene expression is desired, a fragment of the IGF IR cDNA clone of at least about 100 bp and containing the region surrounding the ATG start site is isolated and purified for insertion in the vector. These applications include stimulation of burn and other wound healing, angiogenesis and nerve regeneration.

Where down-regulation of IGF IR gene expression is desired, the XhoI-KpnI fragment of the IGF IR cDNA is used with insertion opposite in orientation to the expression control sequences (as in Example 3 herein). Other portions of the IGF IR cDNA of at least about 100 bp can be substituted. Such applications include prevention of postangioplasty restenosis or atherosclerosis of coronary arteries following cardiac transplant.

Control vectors are those lacking an IGF IR-specific insert. The vector used is a replication-deficient adenovirus vector (Stratford-Perricaudet et al. (1992) *J. Clin. Invest.* 90:626-630; Mastrangeli et al. (1993) *J. Clin. Invest.* 91:225-234) which is passaged in 293 cells (Mastrangeli et al. (1993) supra; Graham et al. (1973) *Virology* 52:456-467) to prepare virus stocks having 1-5 x 10^{10} plague-forming units/ml. Virus is administered hereinbelow.

Example 12 Trans-Catheter Delivery of Oligonucleotides

Domestic Yorkshire pigs (12-15 kg) are used as an animal model in tests of antisense oligonucleotide therapy to prevent postangioplasty restenosis. Pigs are anesthetized with zolazepamin-tiletamine (6.0 mg/kg) in combination with intramuscular rompun (2.2 mg/kg) with 1% nitrous oxide.

The iliofemoral arteries are exposed using sterile surgical procedures, and a double-balloon catheter (C.R. Bard, Inc.) is inserted into the iliofemoral artery as previously described (Nabel et al. (1990) *Science* 249:1285; Nabel et al. (1989) *Science* 244:1342). The proximal balloon is inflated to 500 mmHg, as measured using an on-line pressure transducer, for 5 min. The balloon is then deflated and the catheter is advanced so that the central space between the proximal and distal balloons now

occupi s the region of the previous injury. Both balloons are inflated, and the segment is irrigated with heparinized saline.

The antisense oligonucleotide preparations; antisense oligonucleotide (SEQ ID NO:1) or mismatch oligonucleotide control (SEQ ID NO:3) 1 mg per vessel, in saline, 2 ml volume, are instilled for 20 min in the central space in the catheter.

The catheter is removed, and antegrade blood flow is restored. The arteries are analyzed 21 or 42 days later to determine vascular proliferation and/or restenosis. After appropriate histological preparation, measurements of intimal (I) and medial (M) areas are determined in at least four sections from each artery, and the measurements from each artery are averaged. Comparisons of I/M area ratios for antisense-treated v. mismatch-treated are made using ANOVA with Dunnett's t test (Dunnet, C.W. (1984) *Biometrics* 20:482). Statistical significance is assumed if a null hypothesis could be rejected at the 0.05 level.

Alternatively, in place of oligonucleotides, when inhibition of angiogenesis or vascular muscle proliferation is desired, a recombinant DNA molecule capable of directing the expression of IGF IR-specific antisense RNA and preferably only transiently expressed is administered by the above procedure. Such a recombinant molecule is described in Example 11 herein. The adenovirus vector described therein is replication-deficient and therefore not stably maintained in the tissue. An adenovirus vector can be used at an exemplary dose of about 5×10^9 to 1×10^{10} plaque forming units, in a volume of about 0.7 ml.

Delivery of virus vectors is also described in Guzman et al. (1993) *Circulation* 88:2838-2848; Lemarchand et al. (1993) *Circulation Research* 72:1132-1138. Delivery of oligonucleotides to the interior of blood vessels is described, e.g., in Shi et al. (1994) *Circulation* 90:944-951 and in Simon et al. (1992) *Nature* 359:67-70.

Example 13 Materials

Recombinant human IGF I was kindly provided by Dr. H.P. Guter, CIBA-GEIGY Corp., Summit, NJ [^{3}H]-thymidine (20 Ci/mmole),

[α-^{32}P]UTP (3000 Ci/mmol)], [α-^{32}P]dCTP (3000 Ci/mmol) and ^{125}I-IGF I (~300 μCi/μg), and [^{125}I-Sar^{1}-Ile^{8}]-ang II (2200 Ci/mmol) were obtained from Du Pont-New England Nuclear (Boston, MA). Angiotensin II was purchased from Sigma Chemical Co. (St. Louis, MO).

Referring to SEQ ID NO:8, the cDNA sequence including the nucleotide sequence encoding rat IGF I receptor and SEQ ID NO:9, the deduced amino acid sequence, Applicant notes the following aspects: The coding sequence of the precursor protein extends from nucleotide 46 to nucleotide 4158, including the TGA stop codon within the encoded protein. The signal peptide is from amino acids -30 to -1 of SEQ ID NO:9. The α-subunit begins at amino acid 1. The C-terminus of the α-subunit is generated by protease cleavage at the 4-Arg proteolytic processing site (amino acids 708-711). The cysteine-rich region of the α-subunit is from amino acids 148 to 302. The ß-subunit extends from amino acids 712 to 1340 of SEQ ID NO:9. The ß-subunit transmembrane domain extends from amino acids 908 to 924, and the tyrosine kinase domain within the ß-subunit extends from amino acids 976 to 1084 of SEQ ID NO:9.

With reference to SEQ ID NO:10 and SEQ ID NO:11, the precursor nucleotide sequence and deduced amino acid sequence for human IGF I receptor (from Ullrich et al. (1986) *EMBO Journal* 5:2503-2512) the coding sequence extends from nucleotide 46 to 4149 of SEQ ID NO:10 (including the stop codon). The signal peptide extends from amino acids -30 to -1 of SEQ ID NO:11. The mature α-subunit begins at amino acid 1 and extends to the proteolytic cleavage site at amino acids 707 to 710; the ß-subunit begins at amino acid 711 and extends to amino acid 1337. The ß-subunit transmembrane domain is at amino acids 906 to 929.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Delafontaine, Patrick

(ii) TITLE OF INVENTION: Methods for Regulation of Insulin-like Growth Factor 1 Receptor

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Greenlee and Winner, P.C.
(B) STREET: 5370 Manhattan Circle, Suite 201
(C) CITY: Boulder
(D) STATE: Colorado
(E) COUNTRY: U.S.A.
(F) ZIP: 80303

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO
(B) FILING DATE: 27-SEP-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/317,898
(B) FILING DATE: 04-OCT-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Ferber, Donna M.
(B) REGISTRATION NUMBER: 33,878
(C) REFERENCE/DOCKET NUMBER: 48-94A PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 499-8080
(B) TELEFAX: (303) 499-8089

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCGGAGCCA GACTTCATTC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATGAAGTC TGGCTCCGGA 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCGGTCCCA CTCTTGTTTG 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGCTGCTGA TAGTCGTTGC 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCAACGACTA TCAGCAGCTG 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCCCAAATAA AAGGAATG 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGCAGATTGC CCTTC 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4696 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..4158

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 46..135

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..4155

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTTTTTTTT TTTGAGAAAA GGGAATTTCG TCCCAAATAA AAGGA ATG AAG TCT 54
Met Lys Ser
-30

GGC TCC GGA GGA GGG TCC CCG ACC TCG CTG TGG GGG CTC GTG TTT CTC 102
Gly Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp ly Leu Val Phe Leu
-25 -20 -15

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TCC GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA AT  TGT GGG CCC        150
Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu I  Cys Gly Pro
    -10                 -5                   1                5

GGC ATT GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAA AAC        198
Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn
                10                  15                  20

TGC ACG GTG ATC GAG GGC TTC CTC CAC ATC CTG CTC ATC TCC AAG GCC        246
Cys Thr Val Ile Glu Gly Phe Leu His Ile Leu Leu Ile Ser Lys Ala
            25                  30                  35

GAG GAC TAC CGA AGC TAC CGC TTC CCC AAG CTC ACA GTC ATC ACC GAG        294
Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu
        40                  45                  50

TAC TTG CTG CTG TTT CGA GTG GCC GGC CTC GAG AGC CT  GA GAC CTC        342
Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu
    55                  60                  65

TTC CCG AAC CTC ACA GTC ATC CGT GGC TGG AAA CTC TTC TAC AAT TAC        390
Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr
70                  75                  80                  85

GCA CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAT        438
Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr
                90                  95                  100

AAT CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAC GCT        486
Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala
            105                 110                 115

GAC CTC TGT TAC CTC TCC ACC ATA GAC TGG TCT CTC ATC TTG GAT GCG        534
Asp Leu Cys Tyr Leu Ser Thr Ile Asp Trp Ser Leu Ile Leu Asp Ala
        120                 125                 130

GTG TCC AAT AAC TAC ATT GTG GGG AAC AAG CCC CCA AAG GAA TGT GGG        582
Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly
    135                 140                 145

GAC CTG TGT CCA GGG ACC TTG GAG GAG AAG CCC ATG TGT GAG AAG ACC        630
Asp Leu Cys Pro Gly Thr Leu Glu Glu Lys Pro Met Cys Glu Lys Thr
150                 155                 160                 165

ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAT CGC TGC        678
Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys
                170                 175                 180

CAG AAA ATG TGC CCA AGT GTG TGT GGG AAG CGA GCC TGC ACC GAG AAC        726
Gln Lys Met Cys Pro Ser Val Cys Gly Lys Arg Ala Cys Thr Glu Asn
            185                 190                 195

AAT GAG TGC TGC CAC CCG GAG TGC CTA GGC AGC TGC CAC ACA CCG GAC        774
Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys His Thr Pro Asp
        200                 205                 210

GAC AAC ACA ACC TGC GTG GCC TGC CGA CAC TAC TAC TAC AAA GGC GTG        822
Asp Asn Thr Thr Cys Val Ala Cys Arg His Tyr Tyr Tyr Lys Gly Val
    215                 220                 225

TGC GTG CCT GCC TGC CCG CCT  GC ACC TAC AGG TTC GAG GGC TGG CGC        870
Cys Val Pro Ala Cys Pro Pro Gly Thr Tyr Arg Phe Glu Gly Trp Arg
230                 235                 240                 245

TGT GTG GAC CGG GAT TTC TGC GCC AAC ATC CCC AAC GCC GAG AGC AGT        918
Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Pro Asn Ala  lu Ser Ser
                250                 255                 260
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GAC TCA GAT GGC TTC GTC ATC CAC GAT GGC GAG TGC ATG CAG GAG TGT      966
Asp Ser Asp Gly Phe Val Ile His Asp Gly Glu Cys Met Gln Glu Cys
            265                 270                 275

CCA TCA GGC TTC ATC CGC AAC AGC ACC CAG AGC ATG TAC TGT ATC CCC     1014
Pro Ser Gly Phe Ile Arg Asn Ser Thr Gln Ser Met Tyr Cys Ile Pro
        280                 285                 290

TGT GAA GGC CCC TGC CCC AAG GTC TGC GGC GAT GAA GAA AAG AAA ACG     1062
Cys Glu Gly Pro Cys Pro Lys Val Cys Gly Asp Glu Glu Lys Lys Thr
    295                 300                 305

AAA ACC ATC GAT TCT GTG ACG TCT GCC CAG ATG CTC CAA GGG TGC ACC     1110
Lys Thr Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly Cys Thr
310                 315                 320                 325

ATT TTG AAG GGC AAT CTG CTT ATT AAC ATC CGG CGA GGC AAT AAC ATT     1158
Ile Leu Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile
            330                 335                 340

GCC TCG GAA TTG GAG AAC TTC ATG GGG CTC ATC GAG GTG GTG ACT GGC     1206
Ala Ser Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly
            345                 350                 355

TAC GTG AAG ATC CGC CAT TCC CAT GCC TTG GTC TCC TTG TCC TTC CTG     1254
Tyr Val Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu
        360                 365                 370

AAG AAC CTT CGT CTC ATC TTA GGA GAG GAG CAG CTA GAA GGA AAC TAC     1302
Lys Asn Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr
    375                 380                 385

TCC TTC TAT GTC CTG GAC AAC CAG AAC TTG CAG CAG CTG TGG GAC TGG     1350
Ser Phe Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp
390                 395                 400                 405

AAC CAC CGG AAC CTG ACC GTC AGG TCA GGG AAA ATG TAC TTC GCT TTC     1398
Asn His Arg Asn Leu Thr Val Arg Ser Gly Lys Met Tyr Phe Ala Phe
            410                 415                 420

AAT CCC AAG CTG TGT GTC TCT GAA ATT TAC CGA ATG GAG GAG GTG ACA     1446
Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val Thr
            425                 430                 435

GGA ACA AAG GGA CGG CAG AGC AAA GGA GAC ATA AAC ACC AGG AAC AAC     1494
Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg Asn Asn
        440                 445                 450

GGA GAG CGA GCT TCC TGT GAA AGT GAT GTT CTC CGT TTC ACC TCC ACC     1542
Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu Arg Phe Thr Ser Thr
    455                 460                 465

ACC ACC TGG AAG AAC CGC ATC ATC ATA ACG TGG CAC CGG TAC CGG CCG     1590
Thr Thr Trp Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr Arg Pro
470                 475                 480                 485

CCG GAC TAC CGG GAT CTC ATC AGT TTC ACA GTC TAC TAC AAG GAG GCA     1638
Pro Asp Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys Glu Ala
            490                 495                 500

CCC TTT AAA AAC GTC ACG GAA TAC GAC GGG CAG GAT GCC TGT GGC TCC     1686
Pro Phe Lys Asn Val Thr  lu Tyr Asp Gly Gln Asp Ala Cys Gly Ser
            505                 510                 515

AAC AGC TGG AAC ATG GTG GAC GTG GAC CTG CCT CCG AAC AAG GAG GGG     1734
Asn Ser Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys Glu Gly
        520                 525                 530
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GAG CCT GGC ATT TTG CTG CAT GGG CTG AAG CCC TGG ACC CAG TAT GCA      1782
Glu Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln Tyr Ala
    535                 540                 545

GTC TAT GTC AAG GCT GTG ACC CTC ACC ATG GTG GAA AAC GAC CAC ATC      1830
Val Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile
550                 555                 560                 565

CGT GGG GCC AAA AGT GAA ATC TTG TAC ATT CGC ACC AAC GCT TCA GTT      1878
Arg Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val
                570                 575                 580

CCT TCC ATT CCT CTA GAT GTC CTC TCG GCA TCA AAC TCC TCC TCT CAG      1926
Pro Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln
            585                 590                 595

CTG ATC GTG AAG TGG AAC CCC CCA ACT CTG CCC AAT GGT AAC TTG AGT      1974
Leu Ile Val Lys Trp Asn Pro Pro Thr Leu Pro Asn Gly Asn Leu Ser
        600                 605                 610

TAC TAC ATT GTG AGG TGG CAG CGG CAG CCG CAG GAT GGC TAT CTG TTC      2022
Tyr Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Phe
    615                 620                 625

CGG CAC AAC TAC TGC TCC AAA GAC AAA ATA CCC ATC AGA AAG TAC GCC      2070
Arg His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala
630                 635                 640                 645

GAT GGT ACC ATC GAT GTG GAG GAG GTG ACA GAA AAT CCC AAG ACA GAA      2118
Asp Gly Thr Ile Asp Val Glu Glu Val Thr Glu Asn Pro Lys Thr Glu
                650                 655                 660

GTC TGC GGT GGT GAT AAA GGG CCG TGC TGT GCC TGT CCT AAA ACC GAA      2166
Val Cys Gly Gly Asp Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu
            665                 670                 675

GCT GAG AAG CAG GCT GAG AAG GAG GAG GCT GAG TAC CGT AAA GTC TTT      2214
Ala Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe
        680                 685                 690

GAG AAT TTC CTT CAC AAC TCC ATC TTT GTG CCC AGA CCT GAG AGG AGG      2262
Glu Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Arg
    695                 700                 705

CGG AGA GAT GTC CTG CAG GTG GCT AAC ACC ACC ATG TCC AGC CGA AGC      2310
Arg Arg Asp Val Leu Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser
710                 715                 720                 725

AGG AAC ACC ACG GTA GCT GAC ACC TAC AAT ATC ACA GAC CCG GAA GAG      2358
Arg Asn Thr Thr Val Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu
                730                 735                 740

TTC GAG ACA GAA TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC AAG GAG      2406
Phe Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Lys Glu
            745                 750                 755

AGG ACT GTC ATT TCC AAC CTC CGG CCT TTC ACT CTG TAC CGT ATC GAT      2454
Arg Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Arg Ile Asp
        760                 765                 770

ATC CAC AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC      2502
Ile His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser
    775                 780                 785

AAC TTT GTC TTT GCA AGA ACC ATG CCA GCA GAA GGA GCA GAT GAC ATT      2550
Asn Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile
790                 795                 800                 805
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CCT GGC CCA GTG ACC TGG GAG CCA AGA CCT GAA AAC TCC ATC TTT TTA      2598
Pro Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu
                 810                 815                 820

AAG TGG CCA GAA CCC GAG AAC CCC AAC GGA TTG ATT CTA ATG TAT GAA      2646
Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met Tyr Glu
            825                 830                 835

ATA AAA TAC GGA TCG CAA GTC GAG GAT CAG CGG GAA TGT GTG TCC AGA      2694
Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val Ser Arg
        840                 845                 850

CAG GAG TAC AGG AAG TAT GGA GGG GCC AAA CTT AAC CGT CTA AAC CCA      2742
Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu Asn Pro
    855                 860                 865

GGG AAC TAT ACG GCC CGG ATT CAG GCT ACC TCC CTC TCT GGG AAT GGG      2790
Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly Asn Gly
870                 875                 880                 885

TCG TGG ACA GAT CCT GTG TTC TTC TAT GTC CCA GCC AAA ACA ACG TAT      2838
Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Pro Ala Lys Thr Thr Tyr
                890                 895                 900

GAG AAT TTC ATG CAT CTG ATC ATT GCT CTG CCG GTT GCC ATC CTG CTG      2886
Glu Asn Phe Met His Leu Ile Ile Ala Leu Pro Val Ala Ile Leu Leu
            905                 910                 915

ATT GTG GGG GGC CTG GTA ATC ATG CTG TAT GTC TTC CAT AGA AAG AGG      2934
Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg Lys Arg
        920                 925                 930

AAT AAC AGC AGA TTG GGC AAC GGG GTG CTG TAC GCC TCT GTG AAC CCC      2982
Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val Asn Pro
    935                 940                 945

GAG TAT TTC AGC GCA GCT GAT GTG TAC GTG CCT GAT GAA TGG GAG GTA      3030
Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp Glu Val
950                 955                 960                 965

GCT CGG GAG AAG ATC ACC ATG AAC CGG GAG CTC GGA CAA GGG TCC TTC      3078
Ala Arg Glu Lys Ile Thr Met Asn Arg Glu Leu Gly Gln Gly Ser Phe
                970                 975                 980

GGG ATG GTC TAT GAA GGA GTG GCC AAG GGC GTG GTC AAG GAC GAG CCT      3126
Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp Glu Pro
            985                 990                 995

GAA ACC AGA GTG GCC ATC AAG ACA GTG AAT GAG GCT GCA AGT ATG CGT      3174
Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser Met Arg
        1000                1005                1010

GAG AGA ATT GAG TTT CTC AAC GAG GCC TCA GTG ATG AAG GAG TTC AAC      3222
Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn
    1015                1020                1025

TGT CAC CAT GTG GTC CGG TTG CTG GGT GTA GTA TCC CAA GGC CAG CCC      3270
Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly Gln Pro
1030                1035                1040                1045

ACC CTG GTC ATC ATG GAA CTA ATG ACA CGT GGC GAT CTC AAA AGT TAT      3318
Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys Ser Tyr
                1050                1055                1060

CTC CGG TCT CTA AGG CCA GAG GTG GAG AAT AAT CTA GTC CTG ATT CCT      3366
Leu Arg Ser Leu Arg Pro Glu Val Glu Asn Asn Leu Val Leu Ile Pro
            1065                1070                1075
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CCG AGC TTA AGC AAG ATG ATC CAG ATG GCT GGA GAG ATT GCA GAT GGC      3414
Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala Asp Gly
        1080                1085                1090

ATG GCC TAC CTC AAT GCC AAC AAG TTC GTC CAC AGA GAC CTG GCT GCT      3462
Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala
    1095                1100                1105

CGG AAC TGC ATG GTA GCT GAA GAT TTC ACA GTC AAA ATT GGA GAT TTT      3510
Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe
1110                1115                1120                1125

GGT ATG ACA CGA GAC ATC TAC GAG ACG GAC TAC TAC CGG AAA GGC GGG      3558
Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly
                1130                1135                1140

AAG GGC TTG CTG CCT GTG CGC TGG ATG TCT CCC GAG TCC CTC AAG GAT      3606
Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp
            1145                1150                1155

GGC GTC TTC ACC ACT CAT TCC GAT GTC TGG TCC TTT GGG GTC GTC CTC      3654
Gly Val Phe Thr Thr His Ser Asp Val Trp Ser Phe Gly Val Val Leu
        1160                1165                1170

TGG GAG ATC GCC ACT CTG GCT GAG CAG CCG TAC CAG GGC CTG TCC AAC      3702
Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn
    1175                1180                1185

GAG CAA GTT CTT CGT TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCG      3750
Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro
1190                1195                1200                1205

GAT AAC TGC CCC GAT ATG CTG TTT GAA CTT ATG CGC ATG TGC TGG CAG      3798
Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln
                1210                1215                1220

TAC AAC CCC AAG ATG CGG CCC TCC TTC CTG GAG ATC ATC GGA AGC ATC      3846
Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Gly Ser Ile
            1225                1230                1235

AAG GAT GAG ATG GAG CCC AGT TTC CAG GAG GTC TCC TTC TAC TAC AGC      3894
Lys Asp Glu Met Glu Pro Ser Phe Gln Glu Val Ser Phe Tyr Tyr Ser
        1240                1245                1250

GAG GAG AAC AAG CCT CCA GAG CCG GAG GAG CTG GAG ATG GAG CTG GAG      3942
Glu Glu Asn Lys Pro Pro Glu Pro Glu Glu Leu Glu Met Glu Leu Glu
    1255                1260                1265

CTG GAG CCC GAG AAC ATG GAG AGC GTC CCG CTG GAC CCT TCG GCC TCC      3990
Leu Glu Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser
1270                1275                1280                1285

TCA GCC TCC CTG CCT CTG CCT GAA AGA CAC TCA GGA CAC AAG GCT GAG      4038
Ser Ala Ser Leu Pro Leu Pro Glu Arg His Ser Gly His Lys Ala Glu
                1290                1295                1300

AAC GGC CCT GGC GTG CTG GTT CTC CGT GCC AGT TTT GAT GAG AGA CAG      4086
Asn Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln
            1305                1310                1315

CCT TAC GCT CAC ATG AAT GGG GGA CGC GCC AAC GAG AGG GCC TTG CCT      4134
Pro Tyr Ala His Met Asn Gly Gly Arg Ala Asn Glu Arg Ala Leu Pro
        1320                1325                1330

CTG CCC CAG TCC TCG ACC TGC TGA TCCTCGACCC CGAAGCACGT GCAAACAGTA     4188
Leu Pro Gln Ser Ser Thr Cys  *
    1335                1340
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ACGTGTGTGT GTGTGTGCGT GCGTGCGTGT GTGCTCACTC GGTGGGCGGA GGGGGGAGCA 4248

GGTTGTAACA ATCTATTCAC AAGCCTCCTG TATCTCAGTG GATCTTCAGA ACTGCCCTTG 4308

CTGCCCACGG GAGTCGGCTT CTCTGCAGTA AACACACTTG GGACCTTCCT TTTTTCAATA 4368

TGCAAGCAGC TTTTTTTATT TCCCTACCCA AACCCTTAAC TGACATGGGC CTCTGCAAAC 4428

CTTAATGACA GCTCTTAATA GCAACAGGAC ACTCGAGAAT TGAGTCTCCT CATTCTCTGC 4488

CTTTTCTCTC TTCTGCCTTC CCTCTCTCTC CTCTCCCTTC CCACTTCCAG GCTCTCCTCT 4548

CTTTTCTCCG CTTCCCAGTG GAAAGCCCAG CGGGGAAGCC TTTATTTATT TTTTTTTTAA 4608

ATCAAATTGA CTTAATGGCT GCCCTGGGGC TCCTCATCAC ACCTGCCTGA GCACCATAGG 4668

TCTTTACAAA AAAGAAAGGA AAAAAACC 4696

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1371 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Lys Ser Gly Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp Gly Leu
-30 -25 -20 -15

Val Phe Leu Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile
-10 -5 1

Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg
5 10 15

Leu Glu Asn Cys Thr Val Ile Glu Gly Phe Leu His Ile Leu Leu Ile
20 25 30

Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val
35 40 45 50

Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu
55 60 65

Gly Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe
70 75 80

Tyr Asn Tyr Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile
85 90 95

Gly Leu Tyr Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu
100 105 110

Lys Asn Ala Asp Leu Cys Tyr Leu Ser Thr Ile Asp Trp Ser Leu Ile
115 120 125 130

Leu Asp Ala Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys
135 140 145

Glu Cys Gly Asp Leu Cys Pro Gly Thr Leu Glu Glu Lys Pro Met Cys
150 155 160

Glu Lys Thr Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr
165 170 175

Asn Arg Cys Gln Lys Met Cys Pro Ser Val Cys Gly Lys Arg Ala Cys
180 185 190

Thr Glu Asn Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys His
195 200 205 210

Thr Pro Asp Asp Asn Thr Thr Cys Val Ala Cys Arg His Tyr Tyr Tyr
215 220 225

Lys Gly Val Cys Val Pro Ala Cys Pro Pro Gly Thr Tyr Arg Phe Glu
230 235 240

Gly Trp Arg Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Pro Asn Ala
245 250 255

Glu Ser Ser Asp Ser Asp Gly Phe Val Ile His Asp Gly Glu Cys Met
260 265 270

Gln Glu Cys Pro Ser Gly Phe Ile Arg Asn Ser Thr Gln Ser Met Tyr
275 280 285 290

Cys Ile Pro Cys Glu Gly Pro Cys Pro Lys Val Cys Gly Asp Glu Glu
295 300 305

Lys Lys Thr Lys Thr Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln
310 315 320

Gly Cys Thr Ile Leu Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly
325 330 335

Asn Asn Ile Ala Ser Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val
340 345 350

Val Thr Gly Tyr Val Lys Ile Arg His Ser His Ala Leu Val Ser Leu
355 360 365 370

Ser Phe Leu Lys Asn Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu
375 380 385

Gly Asn Tyr Ser Phe Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu
390 395 400

Trp Asp Trp Asn His Arg Asn Leu Thr Val Arg Ser Gly Lys Met Tyr
405 410 415

Phe Ala Phe Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu
420 425 430

Glu Val Thr Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr
435 440 445 450

Arg Asn Asn Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu Arg Phe
455 460 465

Thr Ser Thr Thr Thr Trp Lys Asn Arg Ile Ile Ile Thr Trp His Arg
470 475 480

Tyr Arg Pro Pro Asp Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr
485 490 495

Lys Glu Ala Pro Phe Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala
500 505 510

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Cys Gly Ser Asn Ser Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn
515                 520                 525                 530

Lys Glu Gly Glu Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr
                535                 540                 545

Gln Tyr Ala Val Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn
            550                 555                 560

Asp His Ile Arg Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn
        565                 570                 575

Ala Ser Val Pro Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser
    580                 585                 590

Ser Ser Gln Leu Ile Val Lys Trp Asn Pro Pro Thr Leu Pro Asn Gly
595                 600                 605                 610

Asn Leu Ser Tyr Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly
                615                 620                 625

Tyr Leu Phe Arg His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg
            630                 635                 640

Lys Tyr Ala Asp Gly Thr Ile Asp Val Glu Glu Val Thr Glu Asn Pro
        645                 650                 655

Lys Thr Glu Val Cys Gly Gly Asp Lys Gly Pro Cys Cys Ala Cys Pro
    660                 665                 670

Lys Thr Glu Ala Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg
675                 680                 685                 690

Lys Val Phe Glu Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro
                695                 700                 705

Glu Arg Arg Arg Arg Asp Val Leu Gln Val Ala Asn Thr Thr Met Ser
            710                 715                 720

Ser Arg Ser Arg Asn Thr Thr Val Ala Asp Thr Tyr Asn Ile Thr Asp
        725                 730                 735

Pro Glu Glu Phe Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp
    740                 745                 750

Asn Lys Glu Arg Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr
755                 760                 765                 770

Arg Ile Asp Ile His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys
                775                 780                 785

Ser Ala Ser Asn Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala
            790                 795                 800

Asp Asp Ile Pro Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser
        805                 810                 815

Ile Phe Leu Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu
    820                 825                 830

Met Tyr Glu Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys
835                 840                 845                 850

Val Ser Arg Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg
                855                 860                 865
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Leu Asn Pro Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser
870 875 880

Gly Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Pro Ala Lys
885 890 895

Thr Thr Tyr Glu Asn Phe Met His Leu Ile Ile Ala Leu Pro Val Ala
900 905 910

Ile Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His
915 920 925 930

Arg Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser
935 940 945

Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu
950 955 960

Trp Glu Val Ala Arg Glu Lys Ile Thr Met Asn Arg Glu Leu Gly Gln
965 970 975

Gly Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys
980 985 990

Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala
995 1000 1005 1010

Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys
1015 1020 1025

Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln
1030 1035 1040

Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu
1045 1050 1055

Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Val Glu Asn Asn Leu Val
1060 1065 1070

Leu Ile Pro Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile
1075 1080 1085 1090

Ala Asp Gly Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp
1095 1100 1105

Leu Ala Ala Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile
1110 1115 1120

Gly Asp Phe Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg
1125 1130 1135

Lys Gly Gly Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser
1140 1145 1150

Leu Lys Asp Gly Val Phe Thr Thr His Ser Asp Val Trp Ser Phe Gly
1155 1160 1165 1170

Val Val Leu Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly
1175 1180 1185

Leu Ser Asn Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu
1190 1195 1200

Asp Lys Pro Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met
1205 1210 1215

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Cys Trp Gln Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile
    1220                1225                1230

Gly Ser Ile Lys Asp Glu Met Glu Pro Ser Phe Gln Glu Val Ser Phe
1235                1240                1245                1250

Tyr Tyr Ser Glu Glu Asn Lys Pro Pro Glu Pro Glu Glu Leu Glu Met
                1255                1260                1265

Glu Leu Glu Leu Glu Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro
            1270                1275                1280

Ser Ala Ser Ser Ala Ser Leu Pro Leu Pro Glu Arg His Ser Gly His
        1285                1290                1295

Lys Ala Glu Asn Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp
    1300                1305                1310

Glu Arg Gln Pro Tyr Ala His Met Asn Gly Gly Arg Ala Asn Glu Arg
1315                1320                1325                1330

Ala Leu Pro Leu Pro Gln Ser Ser Thr Cys  *
                1335                1340
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4989 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..4149

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 46..135

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..4146

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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TTTTTTTTTT TTTTGAGAAA GGGAATTTCA TCCCAAATAA AAGGA ATG AAG TCT          54
                                                  Met Lys Ser
                                                  -30

GGC TCC GGA GGA GGG TCC CCG ACC TCG GTG TGG GGG CTC CTG TTT CTC        102
Gly Ser Gly Gly Gly Ser Pro Thr Ser Val Trp Gly Leu Leu Phe Leu
    -25                 -20                 -15

TCC GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA ATC TGC GGG CCA        150
Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile Cys Gly Pro
-10                 -5                  1                   5
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GGC ATC GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAG AAC          198
Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn
                10                  15                  20

TGC ACG GTG ATC GAG GGC TAC CTC CAC ATC CTG CTC ATC TCC AAG GCC          246
Cys Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile Ser Lys Ala
            25                  30                  35

GAG GAC TAC CGC AGC TAC CGC TTC CCC AAG CTC ACG GTC ATT ACC GAG          294
Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu
        40                  45                  50

TAC TTG CTG CTG TTC CGA GTG GCT GGC CTC GAG AGC CTC GGA GAC CTC          342
Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu
    55                  60                  65

TTC CCC AAC CTC ACG GTC ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC          390
Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr
70                  75                  80                  85

GCC CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC          438
Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr
                90                  95                  100

AAC CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT          486
Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala
            105                 110                 115

GAC CTC TGT TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG          534
Asp Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala
        120                 125                 130

GTG TCC AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG          582
Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly
    135                 140                 145

GAC CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC          630
Asp Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr
150                 155                 160                 165

ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC TGC          678
Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys
                170                 175                 180

CAG AAA ATG TGC CCA AGC ACG TGT GGG AAG CGG GCG TGC ACC GAG AAC          726
Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr Glu Asn
            185                 190                 195

AAT GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC GCG CCT GAC          774
Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser Ala Pro Asp
        200                 205                 210

AAC GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC TAT GCC GGT GTC          822
Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr Ala Gly Val
    215                 220                 225

TGT GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG TTT GAG GGC TGG CGC          870
Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu Gly Trp Arg
230                 235                 240                 245

TGT GTG GAC CGT GAC TTC TGC GCC AAC ATC CTC AGC GCC GAG AGC AGC          918
Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala Glu Ser Ser
                250                 255                 260

GAC TCC GAG GGG TTT GTG ATC CAC GAC GGC GAG TGC ATG CAG GAG TGC          966
Asp Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met Gln Glu Cys
            265                 270                 275
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CCC TCG GGC TTC ATC CGC AAC GGC AGC CAG AGC ATG TAC TGC ATC CCT      1014
Pro Ser Gly Phe Ile Arg Asn Gly Ser Gln Ser Met Tyr Cys Ile Pro
        280                 285                 290

TGT GAA GGT CCT TGC CCG AAG GTC TGT GAG GAA GAA AAG AAA ACA AAG      1062
Cys Glu Gly Pro Cys Pro Lys Val Cys Glu Glu Glu Lys Lys Thr Lys
    295                 300                 305

ACC ATT GAT TCT GTT ACT TCT GCT CAG ATG CTC CAA GGA TGC ACC ATC      1110
Thr Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly Cys Thr Ile
310                 315                 320                 325

TTC AAG GGC AAT TTG CTC ATT AAC ATC CGA CGG GGG AAT AAC ATT GCT      1158
Phe Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile Ala
                330                 335                 340

TCA GAG CTG GAG AAC TTC ATG GGG CTC ATC GAG GTG GTG ACG GGC TAC      1206
Ser Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly Tyr
            345                 350                 355

GTG AAG ATC CGC CAT TCT CAT GCC TTG GTC TCC TTG TCC TTC CTA AAA      1254
Val Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu Lys
        360                 365                 370

AAC CTT CGC CTC ATC CTA GGA GAG GAG CAG CTA GAA GGG AAT TAC TCC      1302
Asn Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr Ser
    375                 380                 385

TTC TAC GTC CTC GAC AAC CAG AAC TTG CAG CAA CTG TGG GAC TGG GAC      1350
Phe Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp Asp
390                 395                 400                 405

CAC CGC AAC CTG ACC ATC AAA GCA GGG AAA ATG TAC TTT GCT TTC AAT      1398
His Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe Ala Phe Asn
                410                 415                 420

CCC AAA TTA TGT GTT TCC GAA ATT TAC CGC ATG GAG GAA GTG ACG GGG      1446
Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val Thr Gly
            425                 430                 435

ACT AAA GGG CGC CAA AGC AAA GGG GAC ATA AAC ACC AGG AAC AAC GGG      1494
Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg Asn Asn Gly
        440                 445                 450

GAG AGA GCC TCC TGT GAA AGT GAC GTC CTG CAT TTC ACC TCC ACC ACC      1542
Glu Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe Thr Ser Thr Thr
    455                 460                 465

ACG TCG AAG AAT CGC ATC ATC ATA ACC TGG CAC CGG TAC CGG CCC CCT      1590
Thr Ser Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr Arg Pro Pro
470                 475                 480                 485

GAC TAC AGG GAT CTC ATC AGC TTC ACC GTT TAC TAC AAG GAA GCA CCC      1638
Asp Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys Glu Ala Pro
                490                 495                 500

TTT AAG AAT GTC ACA GAG TAT GAT GGG CAG GAT GCC TGC GGC TCC AAC      1686
Phe Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala Cys Gly Ser Asn
            505                 510                 515

AGC TGG AAC ATG GTG GAC GTG GAC CTC CCG CCC AAC AAG GAC GTG GAG      1734
Ser Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys Asp Val Glu
        520                 525                 530

CCC GGC ATC TTA CTA CAT GGG CTG AAG CCC TGG ACT CAG TAC GCC GTT      1782
Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln Tyr Ala Val
    535                 540                 545
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TAC GTC AAG GCT GTG ACC CTC ACC ATG GTG GAG AAC GAC CAT ATC CGT      1830
Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile Arg
550                 555                 560                 565

GGG GCC AAG AGT GAG ATC TTG TAC ATT CGC ACC AAT GCT TCA GTT CCT      1878
Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val Pro
                570                 575                 580

TCC ATT CCC TTG GAC GTT CTT TCA GCA TCG AAC TCC TCT TCT CAG TTA      1926
Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln Leu
            585                 590                 595

ATC GTG AAG TGG AAC CCT CCC TCT CTG CCC AAC GGC AAC CTG AGT TAC      1974
Ile Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn Leu Ser Tyr
        600                 605                 610

TAC ATT GTG CGC TGG CAG CGG CAG CCT CAG GAC GGC TAC CTT TAC CGG      2022
Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Tyr Arg
    615                 620                 625

CAC AAT TAC TGC TCC AAA GAC AAA ATC CCC ATC AGG AAG TAT GCC GAC      2070
His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala Asp
630                 635                 640                 645

GGC ACC ATC GAC ATT GAG GAG GTC ACA GAG AAC CCC AAG ACT GAG GTG      2118
Gly Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys Thr Glu Val
                650                 655                 660

TGT GGT GGG GAG AAA GGG CCT TGC TGC GCC TGC CCC AAA ACT GAA GCC      2166
Cys Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu Ala
            665                 670                 675

GAG AAG CAG GCC GAG AAG GAG GAG GCT GAA TAC CGC AAA GTC TTT GAG      2214
Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe Glu
        680                 685                 690

AAT TTC CTG CAC AAC TCC ATC TTC GTG CCC AGA CCT GAA AGG AAG CGG      2262
Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Lys Arg
    695                 700                 705

AGA GAT GTC ATG CAA GTG GCC AAC ACC ACC ATG TCC AGC CGA AGC AGG      2310
Arg Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser Arg
710                 715                 720                 725

AAC ACC ACG GCC GCA GAC ACC TAC AAC ATC ACC GAC CCG GAA GAG CTG      2358
Asn Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu Leu
                730                 735                 740

GAG ACA GAG TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC AAG GAG AGA      2406
Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Lys Glu Arg
            745                 750                 755

ACT GTC ATT TCT AAC CTT CGG CCT TTC ACA TTG TAC GCG ATC GAT ATC      2454
Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Ala Ile Asp Ile
        760                 765                 770

CAC AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC AAC      2502
His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser Asn
    775                 780                 785

TTC GTC TTT GCA AGG ACT ATG CCC GCA GAA GGA GCA GAT GAC ATT CCT      2550
Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile Pro
790                 795                 800                 805

GGG CCA GT  ACC TGG GAG CCA AGG CCT GAA AAC TCC ATC TTT TTA AAG      2598
Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu Lys
                810                 815                 820
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TGG CCG GAA CCT GAG AAT CCC AAT GGA TTG ATT CTA ATG TAT GAA ATA      2646
Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met Tyr Glu Ile
            825                 830                 835

AAA TAC GGA TCA CAA GTT GAG GAT CAG CGA GAA TGT GTG TCC AGA CAG      2694
Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val Ser Arg Gln
        840                 845                 850

GAA TAC AGG AAG TAT GGA GGG GCC AAG CTA AAC CGG CTA AAC CCG GGG      2742
Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu Asn Pro Gly
    855                 860                 865

AAC TAC ACA GCC CGG ATT CAG GCC ACA TCT CTC TCT GGG AAT GGG TCG      2790
Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly Asn Gly Ser
870                 875                 880                 885

TGG ACA GAT CCT GTG TTC TTC TAT GTC CAG GCC AAA ACA GGA TAT GAA      2838
Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr Gly Tyr Glu
                890                 895                 900

AAC TTC ATC CAT CTG ATC ATC GCT CTG CCC GTC GCT GTC CTG TTG ATC      2886
Asn Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val Leu Leu Ile
            905                 910                 915

GTG GGA GGG TTG GTG ATT ATG CTG TAC GTC TTC CAT AGA AAG AGA AAT      2934
Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg Lys Arg Asn
        920                 925                 930

AAC AGC AGG CTG GGG AAT GGA GTG CTG TAT GCC TCT GTG AAC CCG GAG      2982
Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val Asn Pro Glu
    935                 940                 945

TAC TTC AGC GCT GCT GAT GTG TAC GTT CCT GAT GAG TGG GAG GTG GCT      3030
Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp Glu Val Ala
950                 955                 960                 965

CGG GAG AAG ATC ACC ATG AGC CGG GAA CTT GGG CAG GGG TCG TTT GGG      3078
Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly Ser Phe Gly
                970                 975                 980

ATG GTC TAT GAA GGA GTT GCC AAG GGT GTG GTG AAA GAT GAA CCT GAA      3126
Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp Glu Pro Glu
            985                 990                 995

ACC AGA GTG GCC ATT AAA ACA GTG AAC GAG GCC GCA AGC ATG CGT GAG      3174
Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser Met Arg Glu
        1000                1005                1010

AGG ATT GAG TTT CTC AAC GAA GCT TCT GTG ATG AAG GAG TTC AAT TGT      3222
Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn Cys
    1015                1020                1025

CAC CAT GTG GTG CGA TTG CTG GGT GTG GTG TCC CAA GGC CAG CCA ACA      3270
His His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly Gln Pro Thr
1030                1035                1040                1045

CTG GTC ATC ATG GAA CTG ATG ACA CGG GGC GAT CTC AAA AGT TAT CTC      3318
Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys Ser Tyr Leu
                1050                1055                1060

CGG TCT CTG AGG CCA GAA ATG GAG AAT AAT CCA GTC CTA GCA CCT CCA      3366
Arg Ser Leu Arg Pro Glu Met Glu Asn Asn Pro Val Leu Ala Pro Pro
            1065                1070                1075

AGC CTG AGC AAG ATG ATT CAG ATG GCC GGA GAG ATT GCA GAC GGC ATG      3414
Ser Leu Ser Lys Met Ile Gln Met Ala  ly Glu Ile Ala Asp Gly Met
        1080                1085                1090
```

```
GCA TAC CTC AAC GCC AAT AAG TTC GTC CAC AGA GAC CTT GCT GCC CGG      3462
Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg
    1095                1100                1105

AAT TGC ATG GTA GCC GAA GAT TTC ACA GTC AAA ATC GGA GAT TTT GGT      3510
Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly
1110                1115                1120                1125

ATG ACG CGA GAT ATC TAT GAG ACA GAC TAT TAC CGG AAA GGA GGG AAA      3558
Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys
                1130                1135                1140

GGG CTG CTG CCC GTG CGC TGG ATG TCT CCT GAG TCC CTC AAG GAT GGA      3606
Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp Gly
            1145                1150                1155

GTC TTC ACC ACT TAC TCG GAC GTC TGG TCC TTC GGG GTC GTC CTC TGG      3654
Val Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp
        1160                1165                1170

GAG ATC GCC ACA CTG GCC GAG CAG CCC TAC CAG GGC TTG TCC AAC GAG      3702
Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn Glu
    1175                1180                1185

CAA GTC CTT CGC TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCA GAC      3750
Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro Asp
1190                1195                1200                1205

AAC TGT CCT GAC ATG CTG TTT GAA CTG ATG CGC ATG TGC TGG CAG TAT      3798
Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln Tyr
                1210                1215                1220

AAC CCC AAG ATG AGG CCT TCC TTC CTG GAG ATC ATC AGC AGC ATC AAA      3846
Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser Ser Ile Lys
            1225                1230                1235

GAG GAG ATG GAG CCT GGC TTC CGG GAG GTC TCC TTC TAC TAC AGC GAG      3894
Glu Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr Tyr Ser Glu
        1240                1245                1250

GAG AAC AAG CTG CCC GAG CCG GAG GAG CTG GAC CTG GAG CCA GAG AAC      3942
Glu Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu Pro Glu Asn
    1255                1260                1265

ATG GAG AGC GTC CCC CTG GAC CCC TCG GCC TCC TCG TCC TCC CTG CCA      3990
Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser Ser Leu Pro
1270                1275                1280                1285

CTG CCC GAC AGA CAC TCA GGA CAC AAG GCC GAG AAC GGC CCC GGC CCT      4038
Leu Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly Pro Gly Pro
                1290                1295                1300

GGG GTG CTG GTC CTC CGC GCC AGC TTC GAC GAG AGA CAG CCT TAC GCC      4086
Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln Pro Tyr Ala
            1305                1310                1315

CAC ATG AAC GGG GGC CGC AAG AAC GAG CGG GCC CTG CCG CTG CCC CAG      4134
His Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro Leu Pro Gln
        1320                1325                1330

TCT TCG ACC TGC TGA TCCTTGGATC CTGAATCTGT GCAAACAGTA ACGTGTGCGC      4189
Ser Ser Thr Cys  *
    1335

ACGCGCAGCG GGGTGGGGGG GGAGAGAGAG TTTTAACAAT CCATTCACAA GCCTCCTGTA    4249

CCTCAGTGGA TCTTCAGTTC TGCCCTTGCT GCCCGCGGGA GACAGCTTCT CTGCAGTAAA    4309
```

ACACATTTGG GATGTTCCTT TTTTCAATAT GCAAGCAGCT TTTTATTCCC TGCCCAAACC 4369

CTTAACTGAC ATGGGCCTTT AAGAACCTTA ATGACAACAC TTAATAGCAA CAGAGCACTT 4429

GAGAACCAGT CTCCTCACTC TGTCCCTGTC CTTCCCTGTT CTCCCTTTCT CTCTCCTCTC 4489

TGCTTCATAA CGGAAAAATA ATTGCCACAA GTCCAGCTGG GAAGCCCTTT TTATCAGTTT 4549

GAGGAAGTGG CTGTCCCTGT GGCCCCATCC AACCACTGTA CACACCCGCC TGACACCGTG 4609

GGTCATTACA AAAAAACACG TGGAGATGGA AATTTTTACC TTTATCTTTC ACCTTTCTAG 4669

GGACATGAAA TTTACAAAGG GCCATCGTTC ATCCAAGGCT GTTACCATTT TAACGCTGCC 4729

TAATTTTGCC AAAATCCTGA ACTTTCTCCC TCATCGGCCC GGCGCTGATT CCTCGTGTCC 4789

GGAGGCATGG GTGAGCATGG CAGCTGGTTG CTCCATTTGA GAGACACGCT GGCGACACAC 4849

TCCGTCCATC CGACTGCCCC TGCTGTGCTG CTCAAGGCCA CAGGCACACA GGTCTCATTG 4909

CTTCTGACTA GATTATTATT TGGGGGAACT GGACACAATA GGTCTTTCTC TCAGTGAAGG 4969

TGGGGAGAAG CTGAACCGGC 4989

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1368 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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Leu Phe Leu Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile
                -10                 -5                  1

Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg
            5                   10                  15

Leu Glu Asn Cys Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile
        20                  25                  30

Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val
    35                  40                  45                  50

Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu
                55                  60                  65

Gly Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe
            70                  75                  80

Tyr Asn Tyr Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile
        85                  90                  95

Gly Leu Tyr Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu
    100                 105                 110

Lys Asn Ala Asp Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile
115                 120                 125                 130
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Leu Asp Ala Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys
135 140 145

Glu Cys Gly Asp Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys
150 155 160

Glu Lys Thr Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr
165 170 175

Asn Arg Cys Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys
180 185 190

Thr Glu Asn Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser
195 200 205 210

Ala Pro Asp Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr
215 220 225

Ala Gly Val Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu
230 235 240

Gly Trp Arg Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala
245 250 255

Glu Ser Ser Asp Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met
260 265 270

Gln Glu Cys Pro Ser Gly Phe Ile Arg Asn Gly Ser Gln Ser Met Tyr
275 280 285 290

Cys Ile Pro Cys Glu Gly Pro Cys Pro Lys Val Cys Glu Glu Glu Lys
295 300 305

Lys Thr Lys Thr Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly
310 315 320

Cys Thr Ile Phe Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn
325 330 335

Asn Ile Ala Ser Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val
340 345 350

Thr Gly Tyr Val Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser
355 360 365 370

Phe Leu Lys Asn Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly
375 380 385

Asn Tyr Ser Phe Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp
390 395 400

Asp Trp Asp His Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe
405 410 415

Ala Phe Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu
420 425 430

Val Thr Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg
435 440 445 450

Asn Asn Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe Thr
455 460 465

Ser Thr Thr Thr Ser Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr
470 475 480

Arg Pro Pro Asp Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys
485 490 495

Glu Ala Pro Phe Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala Cys
500 505 510

Gly Ser Asn Ser Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys
515 520 525 530

Asp Val Glu Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln
535 540 545

Tyr Ala Val Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp
550 555 560

His Ile Arg Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala
565 570 575

Ser Val Pro Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser
580 585 590

Ser Gln Leu Ile Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn
595 600 605 610

Leu Ser Tyr Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr
615 620 625

Leu Tyr Arg His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys
630 635 640

Tyr Ala Asp Gly Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys
645 650 655

Thr Glu Val Cys Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys
660 665 670

Thr Glu Ala Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys
675 680 685 690

Val Phe Glu Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu
695 700 705

Arg Lys Arg Arg Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser
710 715 720

Arg Ser Arg Asn Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro
725 730 735

Glu Glu Leu Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn
740 745 750

Lys Glu Arg Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Ala
755 760 765 770

Ile Asp Ile His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser
775 780 785

Ala Ser Asn Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp
790 795 800

Asp Ile Pro Gly Pro Val Thr Trp lu Pro Arg Pro Glu Asn Ser Ile
805 810 815

Phe Leu Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met
820 825 830

Tyr Glu Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val
835 840 845 850

Ser Arg Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu
855 860 865

Asn Pro Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly
870 875 880

Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr
885 890 895

Gly Tyr Glu Asn Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val
900 905 910

Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg
915 920 925 930

Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val
935 940 945

Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp
950 955 960

Glu Val Ala Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly
965 970 975

Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp
980 985 990

Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser
995 1000 1005 1010

Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu
1015 1020 1025

Phe Asn Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly
1030 1035 1040

Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys
1045 1050 1055

Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met Glu Asn Asn Pro Val Leu
1060 1065 1070

Ala Pro Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala
1075 1080 1085 1090

Asp Gly Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu
1095 1100 1105

Ala Ala Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly
1110 1115 1120

Asp Phe Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys
1125 1130 1135

Gly Gly Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu
1140 1145 1150

Lys Asp Gly Val Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val
1155 1160 1165 1170

Val Leu Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu
1175 1180 1185

Ser Asn Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp
1190 1195 1200

Lys Pro Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys
1205 1210 1215

Trp Gln Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser
1220 1225 1230

Ser Ile Lys Glu Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr
1235 1240 1245 1250

Tyr Ser Glu Glu Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu
1255 1260 1265

Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser
1270 1275 1280

Ser Leu Pro Leu Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly
1285 1290 1295

Pro Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln
1300 1305 1310

Pro Tyr Ala His Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro
1315 1320 1325 1330

Leu Pro Gln Ser Ser Thr Cys *
1335

I CLAIM:

1. A method for increasing growth factor receptor gene expression in a vertebrate, said method comprising the step of exposing cells of said vertebrate to an ATG-directed sense oligonucleotide, said oligonucleotide comprising at least 14 nucleotides and being capable of stimulating growth factor receptor gene expression.

2. The method of claim 1, wherein a composition comprising an effective amount of an oligonucleotide having an ATG-directed sense nucleotide sequence directed to a growth factor receptor mRNA and being capable of stimulating expression of said gene is applied to a target tissue, with the result that angiogenesis is stimulated in said target tissue.

3. The method of claim 2 wherein said target tissue is affected by peripheral vascular disease.

4. The method of claim 2 wherein said target tissue is heart tissue which has been affected by myocardial infarction.

5. A method of inhibiting vascular smooth muscle cell proliferation in a coronary artery after a heart transplant or after angioplasty in a vertebrate, said method comprising the step of exposing the interior of said artery to a composition comprising an antisense oligonucleotide of at least 14 nucleotides, said oligonucleotide being complementary to a portion of a mRNA for insulin-like growth factor 1 receptor and capable of inhibiting expression of insulin-like growth factor 1 receptor gene expression.

6. A method of inhibiting angiogenesis in a target tissue, said method comprising the step of administering to said target tissue a composition which comprises a

therapeutically effective amount of an IGF IR-specific antisense oligonucleotide or a recombinant nucleic acid molecule which directs the synthesis of an IGF IR-specific antisense RNA in said target tissue and a pharmaceutically effective carrier.

7. The method of any of claims 1-6 wherein said growth factor receptor gene is the insulin-like growth factor I receptor (IGF 1R) gene.

8. The method of any of claims 6-7 wherein antisense oligonucleotide comprises a nucleotide sequence as given in SEQ ID NO:1 or SEQ ID NO:4.

9. The method of any of claims 1-8 wherein said oligonucleotide is a phosphorothioate oligonucleotide, a phosphorodiothioate oligonucleotide a phosphotriester oligonucleotide, a methylphosphonate oligonucleotide, an phosporamidite oligonucleotide, an α-anomer oligonucleotide, and a phosphoroselenoate oligonucleotide.

10. The method of any of claims 1-9 wherein said vertebrate is rat or human.

11. The method of any of claims 1-9 wherein said vertebrate is a species of swine, equine, bovine, ovine, caprine, feline or canine.

12. The method of any of claims 1-5 and 9-12 wherein said ATG-directed sense oligonucleotide comprises a nucleotide sequence as given in SEQ ID NO:2.

13. An oligonucleotide comprising a nucleotide sequence as given in SEQ ID NO:2.

14. A method of inhibiting vascular smooth muscle cell proliferation in a coronary artery after a heart transplant

or after angioplasty in a vertebrate, said method comprising the step of exposing the interior of said artery to a composition comprising a non-naturally occurring DNA molecule having portion capable of expressing an antisense RNA complementary to at least about 100 nucleotides of a Type I insulin-like growth factor receptor mRNA in vascular smooth muscle cells and a pharmaceutically acceptable carrier.

15. The method of claim 14 wherein said non-naturally occurring recombinant DNA molecule is an adenovirus vector which has been genetically engineered to express said antisense oligonucleotide.

16. A non-naturally occurring recombinant DNA molecule comprising a nucleotide sequence encoding mature insulin-like growth factor 1 receptor from rat.

17. The non-naturally occurring recombinant DNA molecule of claim 14 wherein said rat insulin-like growth factor 1 receptor protein (IGF IR) has an amino acid sequence as given in SEQ ID NO: 9.

18. The non-naturally occurring recombinant DNA molecule of claim 17 wherein said nucleotide sequence encoding mature IGF IR (α subunit and ß subunit) of rat is given in SEQ ID NO: 8 from nucleotide 136 to nucleotide 4156.

19. A method of claim 1, said method comprising the step of applying an effective amount of an oligonucleotide having an ATG-directed sense nucleotide sequence directed to a growth factor receptor mRNA and being capable of stimulating expression of said gene and a pharmaceutically acceptable carrier to vertebrate tissue, with the result that one of wound healing, burn healing, bone healing and collateral blood vessel development is stimulated.

FIGURE 1

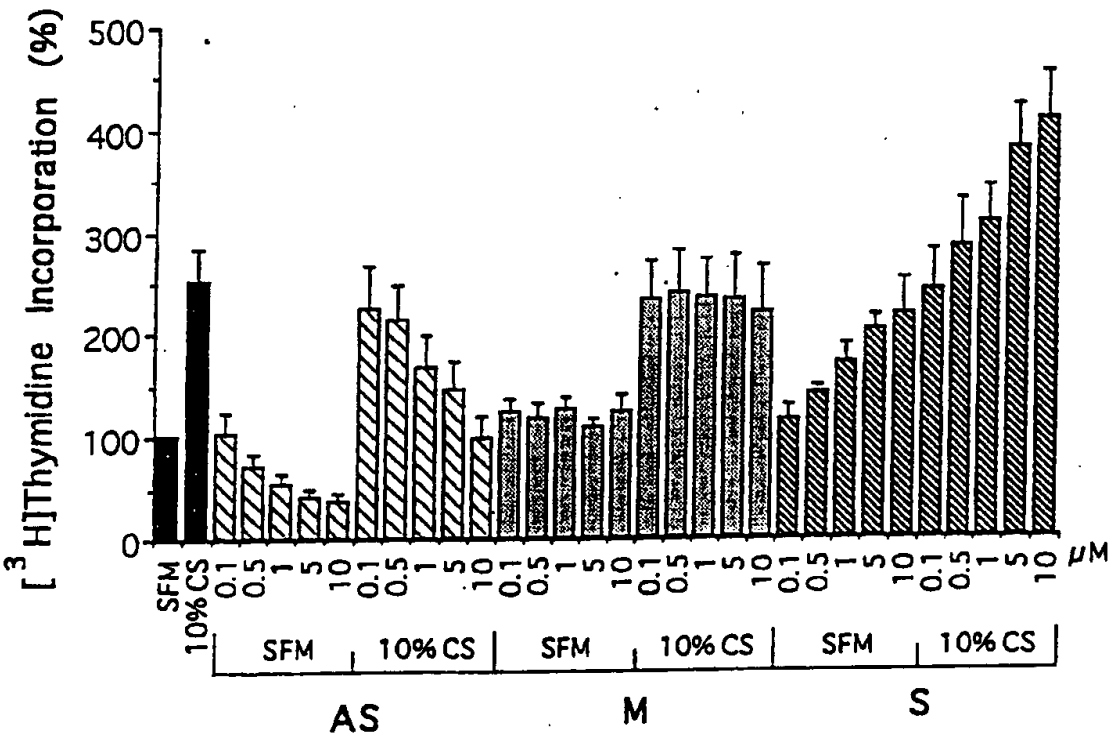

[3H]Thymidine Incorporation (%)
500
400
300
200
100
0
SFM
10% CS
0.1
0.5
1
5
10
μM
SFM
10% CS
SFM
10% CS
SFM
10% CS
AS
M
S

FIGURE 2

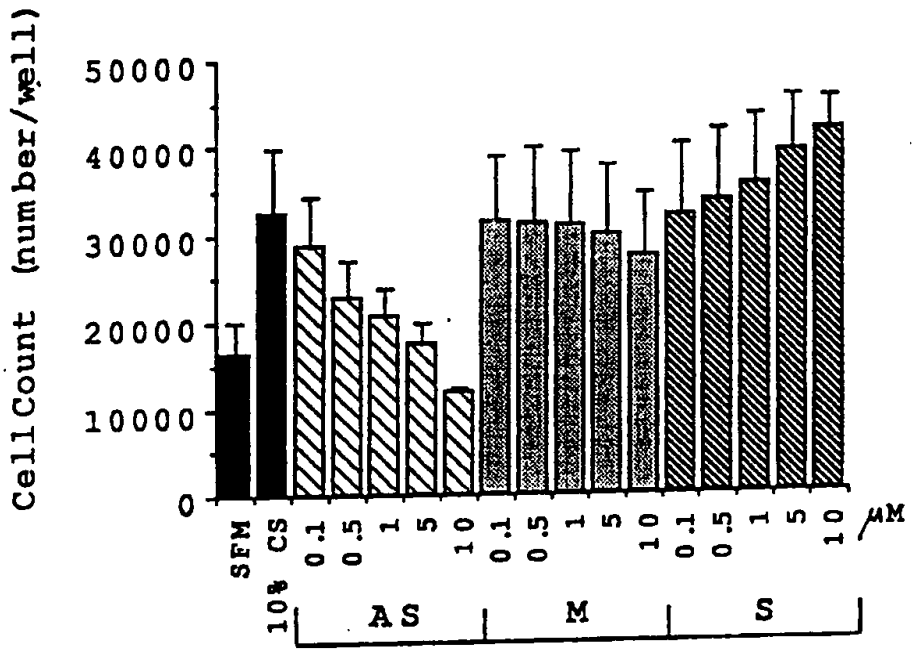

Cell Count (number/well)
50000
40000
30000
20000
10000
0
SFM
10% CS
0.1
0.5
1
5
10
0.1
0.5
1
5
10
0.1
0.5
1
5
10
μM
AS
M
S

FIGURE 3

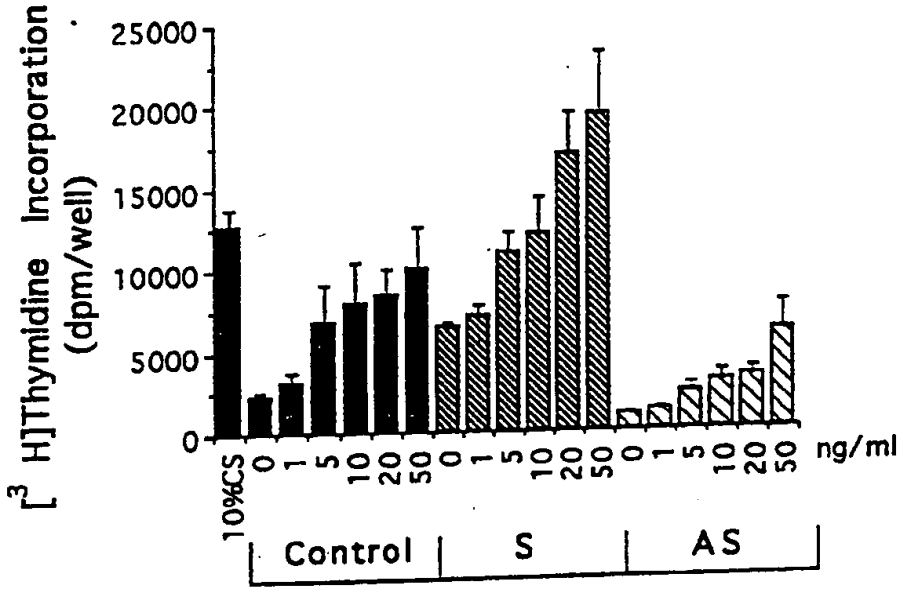

[3 H]Thymidine Incorporation (dpm/well)
25000
20000
15000
10000
5000
0
10%CS
0
1
5
10
20
50
0
1
5
10
20
50
0
1
5
10
20
50
ng/ml
Control
S
AS

FIGURE 4

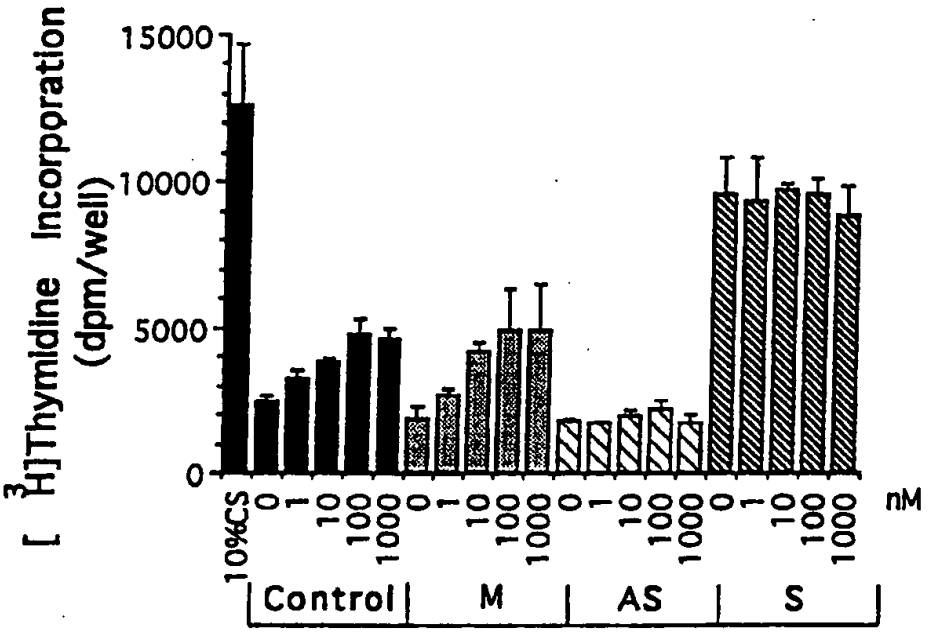

[3H]Thymidine Incorporation (dpm/well)
15000
10000
5000
0
10%CS
0
1
10
100
1000
nM
Control
M
AS
S

FIGURE 5

FIG. 5 A

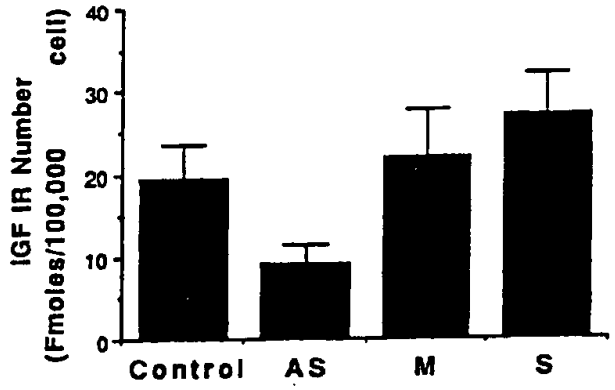


FIG. 5 B

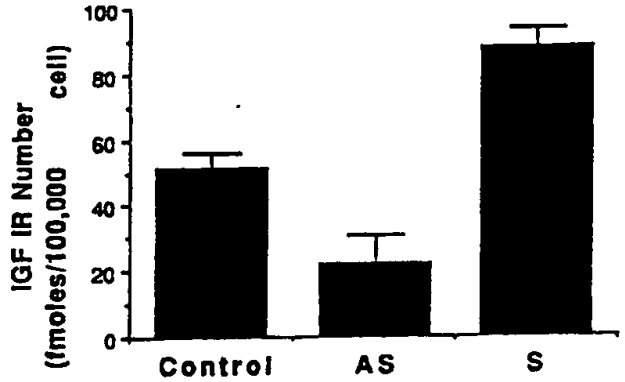


FIG. 5 C

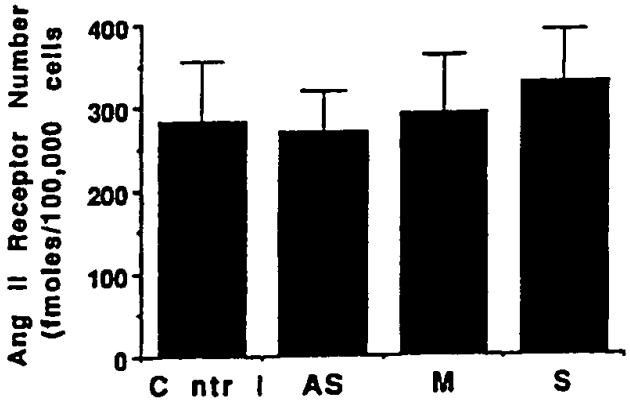


FIGURE 6

FIG. 6 A

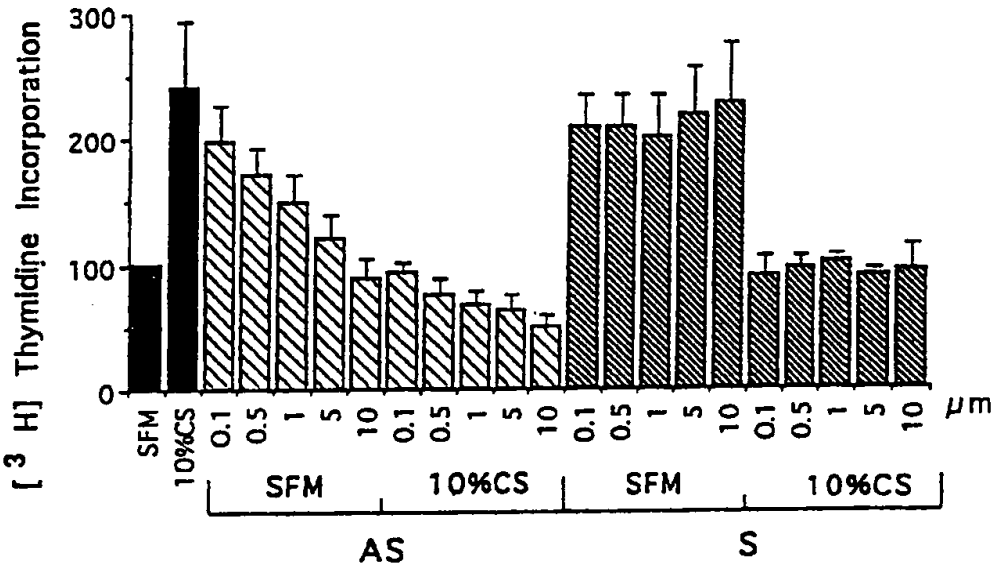


FIG. 6 B

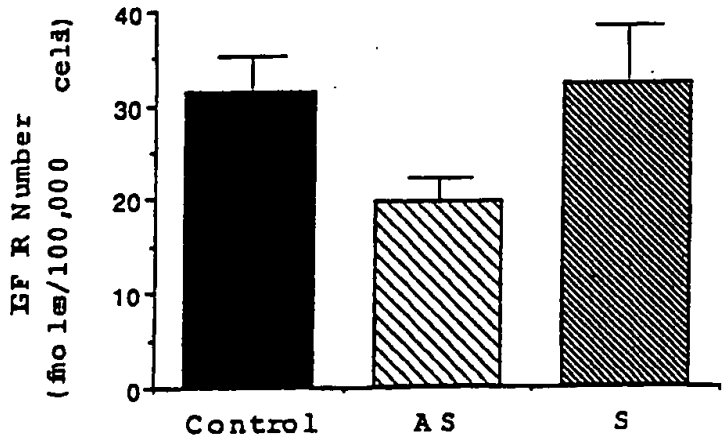


FIGURE 7

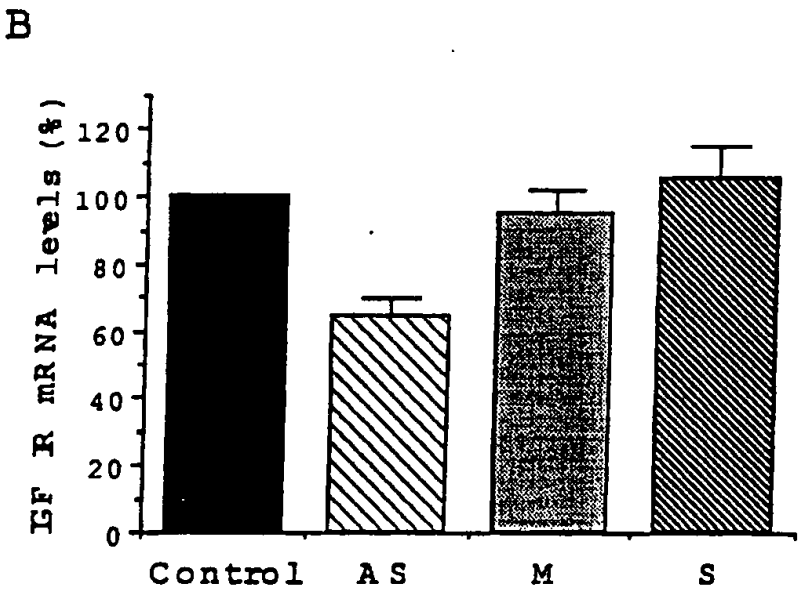
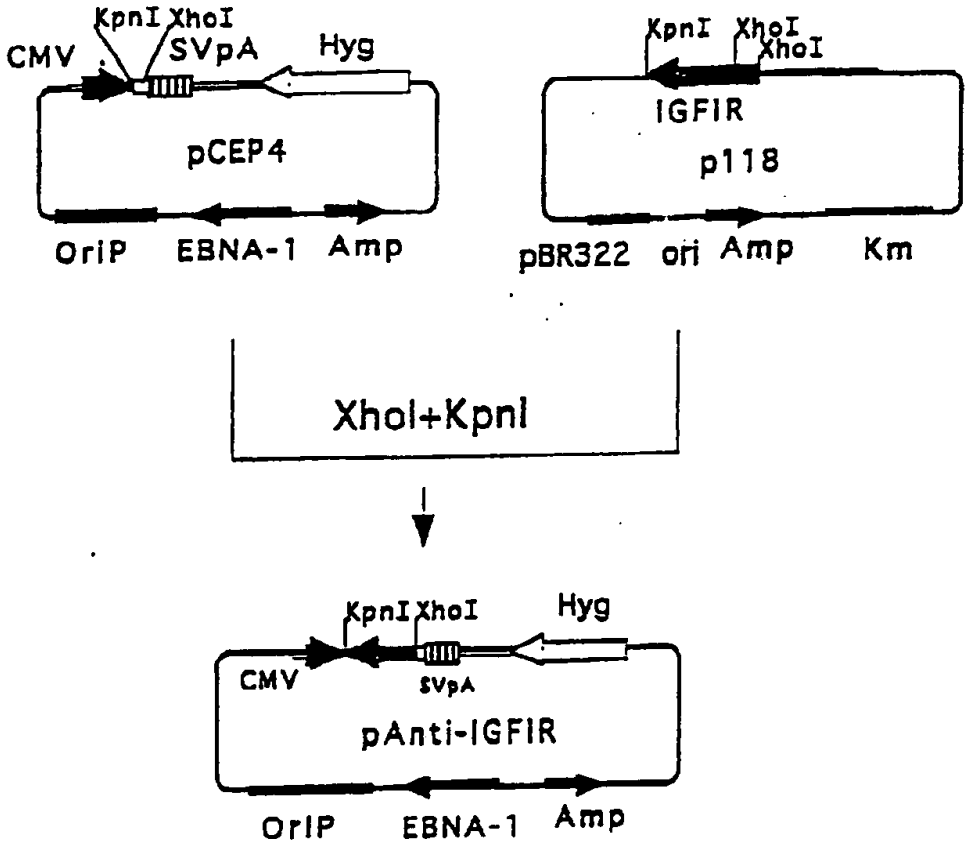


FIGURE 8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CMV
SVpA
Hyg
pCEP4
OriP
EBNA-1
Amp
KpnI XhoI
XhoI
IGFIR
p118
pBR322 ori Amp
Km
XhoI+KpnI
KpnI XhoI
Hyg
CMV
SVpA
pAnti-IGFIR
OriP
EBNA-1
Amp

FIGURE 9

FIG. 9 A

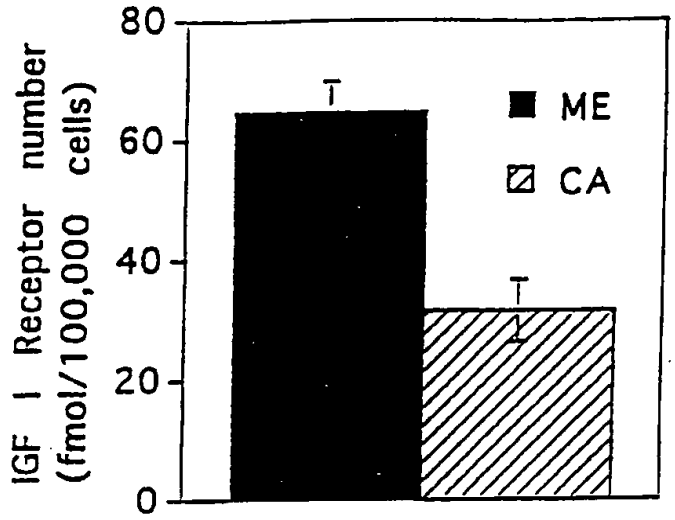


FIG. 9 B

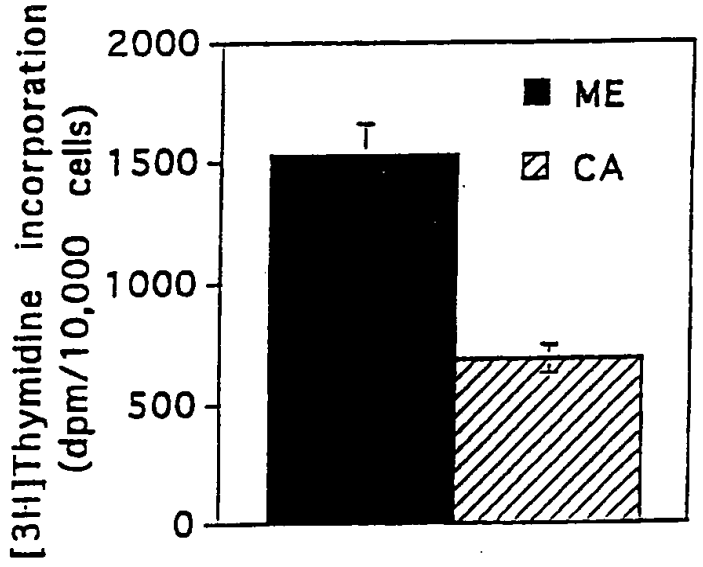
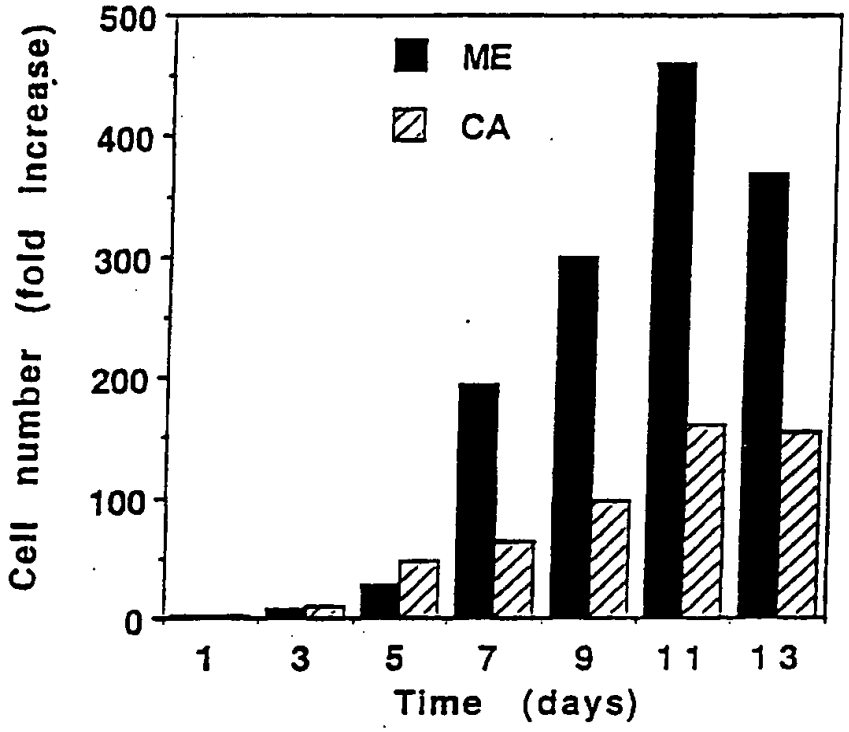


FIGURE 10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Cell number (fold increase)
ME
CA
1
3
5
7
9
11
13
Time (days)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12563

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 31/00; C07H 21/04
US CL : 514/44; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,298,422 (SCHWARTZ ET AL) 29 MARCH 1994, see entire document.	1-15, 19
A,P	US, A, 5,434,134 (GLUCKMAN ET AL) 18 JULY 1995, see entire document.	1-4, 19
Y	WO, A, 93/09236 (BAYLOR COLLEGE OF MEDICINE) 13 MAY 1993, see entire document.	1-15, 19
A,P	WO, A, 94/22486 (THOMAS JEFFERSON UNIVERSITY) 13 OCTOBER 1994, see entire document.	5-18
Y	A. FLYVBJERG et al, "GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR I" published 1993 by WILEY & SONS, (Chichester, UK), pages 159-191, see entire document.	1-15, 19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 DECEMBER 1995

Date of mailing of the international search report
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Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
D. CURTIS HOGUE
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12563

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TRENDS CARDIOVASC MED, Vol. 2, No. 3, issued 1992, Fagin et al, "Growth Factors, Cytokines, and Vascular Injury", pages 90-94, see entire document.	1-15,19
Y	Proceedings of the American Association for Cancer Research, Vol. 33, issued March 1992, Shapiro et al, "Antisense-mediated reduction in insulinlike growth factor-I receptor expression suppresses the malignant phenotype of a human rhabdomyosarcoma", page 354, abstract 2112, see entire document.	5-15
Y	ARTERY, Vol. 18, No. 4, issued 1991, Ferns et al, "The Insulin-Like Growth Factors: Their Putative Role In Atherogenesis", pages 197-225, see entire document.	1-15, 19
Y	CLINICAL RESEARCH, Vol. 42, No. 2, issued 1994, Delafontaine et al, "Antisense Insulin-Like Growth Factor I Receptor Oligonucleotides Inhibit Vascular Smooth Muscle Cell Growth", page 305A, see entire document.	5-15
Y	JOURNAL OF CLINICAL INVESTIGATION, Vol. 94, issued 1994, Shapiro et al, "Antisense-mediated Reduction in Insulin-like Growth Factor-I Receptor Expression Suppresses the Malignant Phenotype of a Human Alveolar Rhabdomyosarcoma", pages 1235-1242, see entire document.	5-15
Y	AMERICAN JOURNAL OF CARDIOLOGY, Vol. 68, issued 04 November 1991, Cercek et al, "Growth Factors in Pathogenesis of Coronary Arterial Restenosis", pages 24C-33C, see entire document.	1-15, 19
Y	CURRENT OPINION IN CARDIOLOGY, Vol. 7, issued 1992, Neville et al, "The molecular biology of vein graft atherosclerosis and myointimal hyperplasia", pages 930-938, see entire document.	1-15, 19
Y	GROWTH REGULATION, Vol. 2, issued 1992, Florini et al, "Induction of Gene Expression in Muscle by the IGFs", pages 23-29, see entire document.	1-15, 19
Y,P	CIRCULATION RESEARCH, Vol. 76, issued 1995, Du et al, "Inhibition of Vascular Smooth Muscle Cell Growth Through Antisense Transcription of a Rat Insulin-Like Growth Factor I Receptor cDNA", pages 963-972, see entire document.	5-18
A	JOURNAL OF LEUKOCYTE BIOLOGY, Vol. 55, issued March 1994, Sunderkotter et al, "Macrophages and angiogenesis", pages 410-422, see entire document.	1-4, 19